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List of Reports issued by the Director of the Onderstepoort Laboratories.

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903-4.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1904-5.*

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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1906-7.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1907-8.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1908-9.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1909-10.*

First Report of the Director of Veterinary Research, August, 1911 *

Second Report of the Director of Veterinary Research, October, 1912.*

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Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918.*

Seventh and Eighth Reports of the Director of Veterinary Research, April, 1918.*

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Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part I, September, 1926.

Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part II, January, 1927.

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Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.

Sixteenth Report of the Director of Veterinary Services and Animal Industry, August, 1930.

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P. J. DU TOIT,
Director of Veterinary Services and Animal Industry.

* Now out of print.

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The Trypanosome Infections of *Glossina pallidipes* in the Umfolosi Game Reserve, Zululand.

(Final Report.)

By A. B. M. WHITNALL, M.Sc., Research Officer (under the Empire Marketing Board), Onderstepoort.

INTRODUCTION.

A preliminary report on this subject was published in the 18th Report of the Director of Veterinary Services and Animal Industry Whitnall (1932). This report contained the percentages of proboscis infections found in *Glossina pallidipes* from several trapping sections of the Umfolosi Game Reserve, between November, 1931, and April, 1932.

In the present report the results of dissection during the period October, 1932, to June, 1933, are given, and a comparison is made of the infections obtained during this period, with those previously recorded. Attempts have been made to follow the possible effect of a reduction in the fly population by means of traps upon the trypanosome infections of the tsetse. The infections found among stray flies have also been briefly considered.

Data pertaining to the vertebrate hosts of the trypanosomes is mentioned in connection with the infections found in fly, the invertebrate hosts.

A short discussion is given upon a peculiar anatomical anomaly which has been noted in this fly.

Further, the salient points, resulting from an investigation into the rôle played by *Stomoxys* as a possible transmitter of Nagana, are recorded in an appendix.

GENERAL.

(1) *Material.*—Work of this nature is largely determined by such external factors, as weather conditions and the number of live flies obtainable.

In all cases the flies taken for dissection have been taken from a standard object, the " Harris " trap.

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In the preliminary report, the flies were also taken from the " Harris " traps in the following sections of the Reserve : Siyembeni, A.5, Domba, A.4, Dadetu, A.3, Mhluzi, B.5, and Mbuzana, B. 1. In the present report the flies have been taken from the traps in Siyembeni, A.5, Mhluzi, B.5, and Dengeza, B.4 only.

The Sections A.5 and B.4 were selected for the following reasons : —(1) The nearest possible continuous data of infection could be obtained from A.5, a section in which trapping has been continuous. (2) In order that the possible effect of the diminution of the fly population upon infections might be followed. (3) Section B.4 was selected because the fly density was greater and material readily obtainable. As trapping had been abandoned since December, 1931, the flies from this section were used as a control against those from A.5, an operating section.

Harris (1932) mentions the unfortunate break in the trapping operations at this period.

(2) *Condition of Flies.*—The condition of the flies examined is based upon external observations and " feel ". 50 per cent. to 60 per cent. of the females are regarded as pregnant, according to the distension of the abdomen. If, however, the females had been completely dissected and examined for developing larvae, it is felt that the percentage of pregnancies would have been higher.

It will be seen in Tables II and III that young flies pale in colour and soft to touch vary in prevalence from month to month.

(3) *Sex Ratio.*—The sex ratio in the various sections is given in Tables II, III and IV.

In all cases there is a predominance of females amongst the flies examined. This predominance is due to the fact that the traps have a greater attraction for females than for males.

Where fly is scarce the percentage of females appears to be relatively low (62 per cent. to 64 per cent.), while on the other hand, where fly is denser the percentage of females appears to be relatively high (71 per cent. to 77 per cent.).

DISCUSSION.

(1) A COMPARISON OF THE INFECTIVITY OF *G. pallidipes* OVER A PERIOD OF TWO YEARS.

Table I gives a comparison between the two periods November, 1931, to April, 1932, and October, 1932, to June, 1933. The infections from the different sections are given in aggregate form.

TABLE I.

The Trypanosome Infections Found in G. pallidipes from Various Localities in the Umfolosi Game Reserve. Given in Aggregate Form.

Date.	No. of Flies Examined.	Male.	Female.	Flies Infected.			Percentage of Flies Infected.										
				Total.	Male.	Female.	Total.	Male.	Female.	*T. vivax.*	*T. vivax.* %	*T. congolense.*	*T. congolense.* %	*T. brucei.*	*T. brucei.* %	Mixed.	Mixed. %
Nov., 1931, to April, 1932.	1,606	423	1,183	55	14	41	3·42	3·31	3·46	1·55	45·4	0·89	25·5	0·24	7·6	0·74	21·8
Oct., 1932, to June, 1933..	1,129	320	809	58	22	36	5·14	6·87	4·45	2·04	39·7	1·86	36·2	0·62	12·1	0·62	12·1

The flies were collected over a wide area, extending from the junction of the Black and White Umfolosi Rivers (Section A.5), to the North-western Section of the Reserve (B.1).

Comparing the infections of the first period with those of the latter, the percentage of flies infected appears to have increased from 3·42 per cent. to 5·14 per cent. There also appears to have been an increase in the percentage of each sex infected.

In the first period the percentage of males and females infected was approximately the same (3·31 per cent. males and 3·46 per cent. females). In the latter period more males than females showed infection (6·87 per cent. males and 4·45 per cent. females).

An increase in the occurrence of each species of trypanosome appears also to have occurred. *T. vivax* was the predominating infection in the first period, representing 45 per cent. of the total infections, or 1·55 per cent. tsetses harboured this parasite. *T. congolense* represented 25 per cent. of the total infections, or 0·89 per cent. flies showed this infection.

T. vivax was still the predominating infection during the latter period, representing 39·7 per cent. of the total infections and occurring in 2·04 per cent. flies. *T. congolense* occurred in 1·86 per cent. flies.

It will be seen that infections of *T. brucei* appear also to have increased during the latter period from 7·6 per cent. to 12·1 per cent. of the total infections. The occurrence of this trypanosome in the fly increased from 0·24 per cent. to 0·62 per cent.

In the same period the percentage of mixed infections appears to have slightly decreased, i.e. from 0·74 per cent. to 0·62 per cent. flies infected. They have occurred as a combination of *T. vivax* and *T. congolense*, and *T. vivax* and *T. brucei*.

In considering the significance or interpretation of these results, consideration must be given to the influence of certain external factors. (1) For example, trapping of the flies has been carried on continuously in the eastern sections of the Reserve and has greatly reduced the fly population. (2) In the western sections trapping was abandoned from December, 1931. (3) Game and its movements is also a factor which must have great influence on the trypanosome infections of the fly. Unfortunately this factor cannot be accurately recorded even by continuous observation.

(2) A COMPARISON OF THE INFECTIONS IN THE TWO SECTIONS A.5 AND B.4.

Due to the game reduction and trapping of the tsetse in the Umfolosi Game Reserve, no true infectivity of *G. pallidipes* under normal undisturbed conditions, is obtainable, for comparison.

An attempt can, however, be made to compare the infectivity of fly under the nearest possible normal conditions, with those which may be considered as decidedly abnormal.

There was considerable variation in the local conditions in the two Sections A.5 and B.4.   Section B.4 was in the abandoned area and consequently fly conditions were returning gradually to normal. Section A.5, on the other hand, was in the area of continuous operation and conditions regarding fly density were abnormal, due to intensive trapping.

The flies from Section B.4 were taken from three of the four check traps, which had been put into operation, for the purpose of gaining knowledge regarding the fly density in this section after abandonment.   The fly was found to be relatively dense as compared with the density in the operating area (60·2 flies per trap per day, October, 1932).

Numerous field traps provided the flies from Section A.5.   The fly density was very low (1·8 flies per trap per day, October, 1932).

The results of proboscis examinations from these two localities are given in Tables II and III.   It is thus possible to make a comparison over the nearest possible similar periods of time.

The percentage of flies infected will be seen to vary in the two localities.

At Section B.4 during October, 1932, 7·6 per cent. flies were infected and at Section A.5, 3·5 per cent.   In January, 1933, Section B.4 gave 5·2 per cent. flies infected, while Section A.5 gave 4·4 per cent.   The infection for April was 5·8 per cent. and for May 8 5 per cent. in Section B.4, while in Section A.5 it was 3·7 per cent. and 5·3 per cent. for the same periods.

Further, it will be seen in the tables that the occurrence of the different species of trypanosome appears to vary at the two localities.

The infection most commonly found in Section A.5 was *T. congolense*, while in Section B.4 *T. vivax* appeared to be most prevalent. In Section B.4 *T. brucei* frequently occurs, but this infection has only once been recorded from A.5.

A point of interest is the percentage of males and females which showed infection, and this also seems to vary over similar periods of time in the two localities.   As the trapping of the fly might have had an influence on this aspect of the trypanosome infections, this variation has been discussed later with reference to one particular section.

It is remarkable that these variations have occurred in localities about five miles apart, and they appear to indicate amongst other things, that the flies do not pass with any regularity from one focus to another.

(3) Variation of Infectivity from Month to Month.

An analysis of the results presented in Tables II and III indicates further that the infectivity varies in one locality from month to month.

TABLE II.

The Proboscis Infections of G. pallidipes taken at Different Intervals from the Same Locality—Siyembeni, A.5.

Date.	Locality.	No. of Flies Examined.	Males.	Females.	Females.	Young Flies.	Young.	Infections.						% Flies Infected with and % Occurrence of each Species of Trypanosome.				Percentage Flies Infected.		
								T. vivax.	T. congolense.	T. brucei.	Mixed Infections. T. vivax and T. congolense.	Mixed Infections. T. vivax and T. brucei.	Total.	T. vivax.	T. congolense.	T. brucei.	Mixed.	Total.	Male.	Female.
14/10/32 to 1/11/32	Siyembeni, A. 5	141	53	88	% 62	15	% 10·6	—	5	—	—	—	5	—	3·5 100	—	—	3·5	9·4	—
28/1/33 to 1/8/33	Siyembeni, A. 5	90	32	58	64	12	13·3	2	1	1	—	—	4	2·2 50	1·1 25	1·1 25	—	4·4	12·5	—
11/4/33 to 12/5/33	Siyembeni, A. 5	108	29	79	73	1	0·93	2	2	—	—	—	4	1·9 50	1·9 50	—	—	3·7	3·5	3·8
29/5/33 to 22/6/33	Siyembeni, A. 5	73	25	48	66	7	9·6	1	2	—	1	—	4	1·4 25	2·7 50	—	1·4 25	5·3	8·0	4·2

TABLE III.

The Proboscis Infections of G. pallidipes taken at Different Intervals from the same Locality—Dengeza, B.4.

Date.	Locality.	No. of Flies Examined.	Males.	Females.	Females. %	Young Flies.	Young. %	Infections. T. vivax.	T. congolense.	T. brucei.	Mixed: T. vivax and T. congolense.	Mixed: T. vivax and T. brucei.	Total.	% Files Infected with and % Occurrence of each Species of Trypanosome. T. vivax.	T. congolense.	T. brucei.	Mixed.	Percentage Files Infected. Total.	Male.	Female.
2/10/32 to 11/10/32	Dengeza, B. 4	158	46	112	71	18	11·3	9	2	—	1	—	12	5·7 / 75	1·3 / 16·7	— / —	0·6 / 8·3	7·6	2·2	9·8
7/11/32 to 8/12/32	Dengeza, B. 4	175	45	130	74	5	2·9	1	4	1	—	1	7	0·6 / 14·3	2·2 / 57·1	0·6 / 14·3	0·6 / 14·3	4·0	2·2	4·6
23/1/33 to 26/1/33	Dengeza, B. 4	97	25	72	74	6	6·2	1	1	1	2	—	5	1·0 / 20	1·0 / 20	1·0 / 20	2·1 / 40	5·2	4·0	5·6
7/4/33 to 13/4/33	Dengeza, B. 4	120	28	92	77	1	0·83	4	—	3	—	—	7	3·3 / 57	— / —	2·5 / 43	— / —	5·8	10·7	4·3
15/5/33 to 11/6/33	Dengeza, B. 4	59	14	45	76	1	1·7	1	1	1	2	—	5	1·7 / 20	1·7 / 20	1·7 / 20	3·4 / 40	8·5	14·3	6·7

For example, at Section B.4 (Table III) for October and November, 1932, and for January, April and May, 1933, the percentages of flies infected were 7·6 per cent., 4·0 per cent., 5·2 per cent., 5·8 per cent., and 8·5 per cent., respectively.

There is further an apparent variation in the occurrence of the several trypanosomes. A predominance of *T. vivax* appears at certain periods, while at others *T. congolense* is more frequent. In regard to *T. brucei* it occurred from November, 1932, to June, 1933, in this section.

Similarly, at Section A.5 (Table II) there were fluctuations of infection from month to month.

From these results and those mentioned in the preliminary report, the proboscis infections of tsetses in Zululand appear to occur throughout the year, while fluctuating in frequency from month to month.

No attempt has been made in this work to determine the percentage of mature and immature infections. However, in 87 per cent. of the infected flies the hypopharynx was found positive, and from this it would seem safe to assume that a large majority of the infected flies harboured trypanosomes of the infective form, and were capable of transmitting the disease.

(4) POSSIBLE EFFECT OF REDUCING THE FLY POPULATION BY TRAPPING UPON THE TRYPANOSOME INFECTIONS.

Table IV gives the total percentage of infections discovered, together with the percentage of males and females infected, and also the percentage of females examined from Section A.5 for eight months.

The daily density per trap is also given for the months where records are obtainable.

In nature the percentage of males and females in *Glossina* is probably 50-50 per cent., yet the " Harris " trap continuously captures more females than males. Further, as the fly population dwindles, the percentage of females captured becomes less.

TABLE IV.

The Trypanosome Infections of G. pallidipes *from One Locality Siyembeni A.5, showing the Possible Effect of Reducing the Fly Population upon these Infections.*

Date.	Females.	Percentage Flies Infected.			Remarks.
		Males.	Females.	Total.	
12/12/31 to 17/12/31	% 68	4·2	2·0	2·7	Severe drought. Fly dense. 18·1 flies per trap per day.
24/1/32 to 3/2/32	82	—	3·7	3·0	Severe drought. Fly dense. No density records.
8/3/32 to 20/3/32	87	2 3	3·6	3·4	Heavy rains, 20–22 Feb. Fly dense. No records.
3/4/32 to 6/4/32	87	-	3·8	3·3	Fly dense. 5·4 flies per trap per day.
14/10/32 to 1/11/32	62	9·4	--	3 5	Fly scarce. 1·8 flies per trap per day.
28/1/33 to 1/3/33	64	12·5	—	4·4	Fly scarce. 0·4 flies per trap per day.
11/4/33 to 12/5/33	73	3·5	3·8	3·7	Fly scarce. 0·4 flies per trap per day.
29/5/33 to 22/6/33	66	8·0	4·2	5·3	Fly scarce. 0·4 flies per trap per day.

This is clearly shown in the table. During the first period (December, 1931, to April, 1932), fly was still comparatively dense in Section A.5 (18·1 flies per trap per day December, 1931) and the percentage of females was relatively high. In the latter period fly became scarcer (0·4 flies per trap per day June, 1933) as trapping proceeded and the female percentage dropped.

These facts appear to have an influence on the infections. During November, 1932, to June, 1933, when the fly population had been greatly reduced, those flies captured in Section A.5 showed abnormal conditions in regard to infection, assuming that the findings for the former period can be accepted as normal.

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When fly was comparatively dense in the Umfolosi Reserve, i.e. between November, 1931, and April, 1932, it was found that 3·31 per cent. males and 3·42 per cent. females were infected in 1,606 flies examined (see Table I).

Duke (1930) has shown that with *G. palpalis* and *T. gambiense* and *T. rhodesiense* there is a slightly higher percentage of females than males infected with the flagellates.

Thus the two sexes of *Glossina* appear to be equally susceptible to infection with Trypanosomes. Males and females live on the same food, and the chances of acquiring infection must therefore be equal.

Bearing in mind the above remarks, some facts of extraordinary interest appear in the table.

In March, 1932, when the fly population was comparatively dense (approximately 5·4 flies per trap per day) and the female percentage of flies dissected was 87 per cent., the infections were 2·3 per cent. males and 3·6 per cent. females. In April, 1932, with approximately similar density, the percentage of females examined was 87 per cent. No males were infected and 3·8 per cent. females.

In November, 1932, when the density had fallen to 1·8 flies per trap per day, the percentage of females examined was 62, and the infections were 9·4 per cent. males, but no females. In February, 1933, when the density had fallen as low as 0·4, 12·5 per cent. males were infected, but no females. Later in May the infections were 8·0 per cent. males and 4·2 per cent. females.

Similarly at Section B.4 as the fly population became less dense (60 flies per trap per day in October, 1933, to 17 in June, 1933, using four check traps as basis) so the percentage of males infected increased from 2·2 per cent. to 14·3 per cent. The percentage of females caught has, however, remained the same.

Minor exceptions occur, but the general tendency is as follows: As the fly population has become less dense, the percentage of flies infected has increased. The increase has occurred in the number of males infected.

The fly population at Section A.5 therefore, when greatly reduced by trapping, has a predominance of males with a high infectivity.

It is possible that this remarkable phenomenon might be due to the greater attraction of the traps for females, and in such cases young females would be picked up more rapidly than males. Thus the male portion of the population in a particular section might show higher infectivity than the female, due to the fact that the males when they happen to be caught, have had a longer period in which to acquire infection.

(5) STRAY FLIES AND THEIR INFECTIONS.

Three traps were erected in the southern buffer zone, within a radius of two miles from camp, to determine the density of fly in this locality. Some of the flies caught have been examined for infections.

It is of interest that the " Harris " trap was capable of catching tsetses where the population was extremely sparse. Doubtless the trap would be a most useful weapon when conducting a fly survey.

Fly was present in small numbers in the vicinity of camp. They appeared to be the merest stragglers from the main body of fly and were only trapped at irregular intervals. From 21st November, 1932, to 10th February, 1933, the three traps caught twenty-five flies, fourteen males and eleven females.

Four females were pregnant, two in advanced stages. The occurrence of pregnant females which are about to deposit larvae, probably has some bearing upon the young flies amongst the stragglers. One tsetse, a young female, was caught at the Nseleni drift, eleven miles from the Game Reserve.

Eighteen flies have been dissected and examined from outside the Game Reserve and four were infected. The numbers are small, but in proportion the percentage infected appears high (22·2 per cent.). Once again, where the fly density was low the percentage of males infected was much greater than the percentage of females infected.

The infections found comprise three *T. congolense* and one *T. brucei.* It thus appears that both of the most pathogenic infections are being carried from their source, the Game Reserve. Though the numbers are small the fact of infected flies straying far afield is one of importance, and has some bearing upon outbreaks of Nagana.

TRYPANOSOMES OF GAME IN ZULULAND.

This work would hardly be complete without some mention of the available data pertaining to the vertebrate host of the trypanosome.

Four workers, Bruce (1895 and 1903) Mitchell (1914), Curson (1921) and Neitz (1931 and 1933 *) have investigated the reservoirs of Nagana infection amongst the game animals of Zululand. Neitz (1931) gives some details regarding the earlier investigators.

His recent investigations which alone will be considered here, amount briefly to the following:—

Two blood, two spleen, and two gland smears from each of 616 game animals have been examined microscopically for blood parasites.

These 616 animals, including zebra, bushbuck, duiker, reedbuck, kudu, warthog, steenbuck, klipspringer, blue wildebeest, waterbuck, and inyala, represent some of the 26,000 head which were shot during the period from May, 1929, to November, 1930, in the Umfolosi buffer zones (Harris, 1932).

Infection was found in seven instances. These comprised four *T. vivax* and three *T. congolense,* and were recorded from four bushbuck, two kudu, and one zebra.

Before considering these results emphasis must be laid on the following factors:—(1) The periodicity with which trypanosomes might appear in the peripheral blood. (2) The technique employed. (3) The time that expired after death before the slides were taken. These three factors have tremendous importance in regard to the frequency with which trypanosomes are encountered in stained preparations.

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* This paper is now in press.
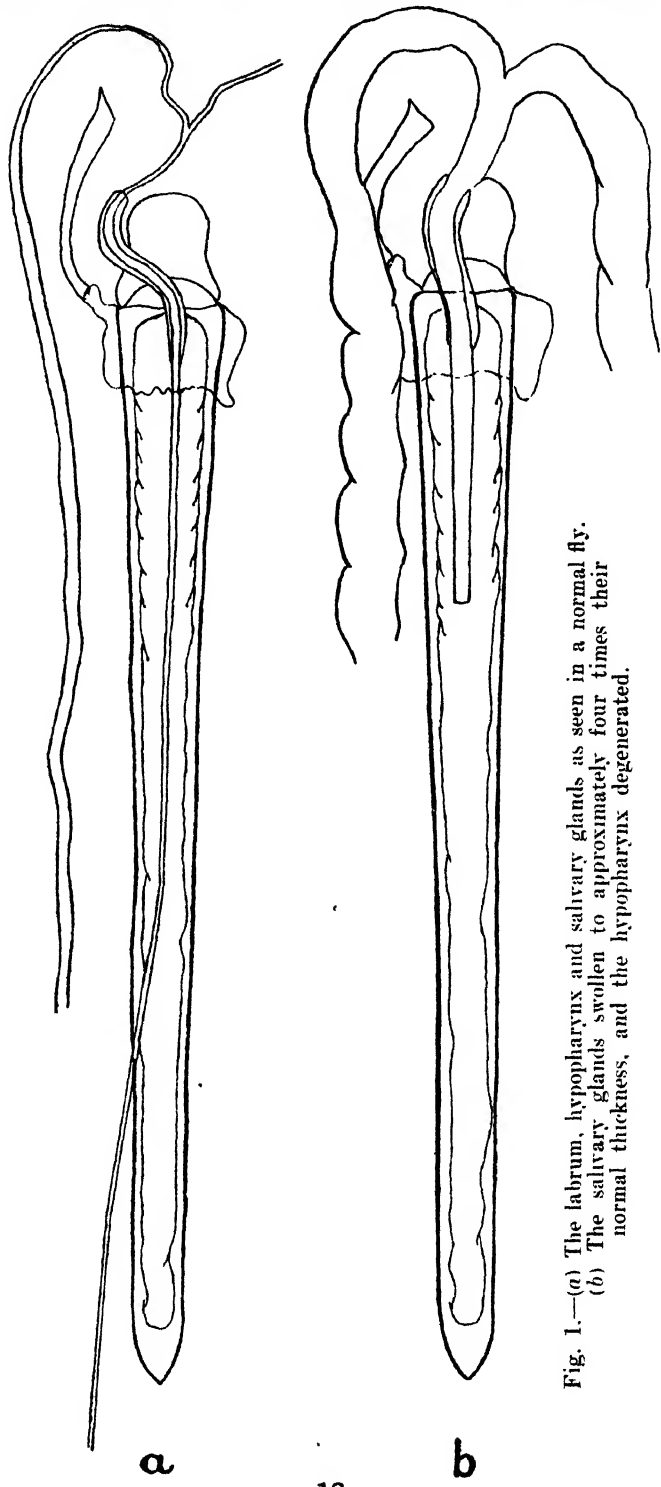
Fig. 1.—(a) The labrum, hypopharynx and salivary glands as seen in a normal fly. (b) The salivary glands swollen to approximately four times their normal thickness, and the hypopharynx degenerated.

a   b

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The percentage of game infected, determined from the above fragmentory data appears to be 1·14 per cent., while the tsetse fly population on the average appears to be infected to the extent of 4·13 per cent.

The complicating factors are so numerous that no attempt will be made to correlate these happenings. Nevertheless it is of interest to note that *T. vivax* and *T. congolense* have been recorded from game, and the former appears to be more common. *T. brucei* has not been recorded since Bruce found it in 1895 and 1903, at which time the three species of trypanosomes had not been differentiated.

In the fly *T. vivax* and *T. congolense* frequently occur and the former is the most common. *T. brucei*, on the other hand, is a fairly rare parasite.

AN ANATOMICAL ANOMALY IN "G. PALLIDIPES".

While engaged on fly dissection a peculiar anomaly in the structure of the salivary glands and hypopharynx of this tsetse was noted. In 1,129 flies examined, 32, that is 2·8 per cent., showed a distinct thickening of the salivary glands. It was most noticeable and was detected as soon as the salivary glands were displayed. Furthermore, in each case where the salivary glands were thickened the hypopharynx was invariably degenerated.

Normally, the hypopharynx, a continuation of the salivary duct, is a long slender tube extending beyond the tip of the labrum, while the salivary glands are slender thread-like organs. Fig. 1 (*a*).

In the cases now under discussion the glands may be swollen to more than four times their normal thickness, and show convulutions, without sign of infection. The hypopharynx is broad and stumpy, being approximately three or four times the normal breadth, and sometimes less than one-tenth of the normal length. Fig. 1 (*b*).

The anomaly has been found in males, females and young flies. That such flies can feed, and live for some time, is borne out by the fact that mature trypanosome infections of *T. brucei* have been found in some of them, indicating that the flies have lived at least three weeks.

The point of interest arises in connection with the trypanosome infections.

Lloyd and Johnson have stated that in the case of *T. brucei* the hypopharynx is used only as a passage for mature trypanosomes from the salivary glands. With *T. congolense* and *T. vivax* pre-infective forms are said to enter the hypopharynx and there become infective. The infective forms accumulate.

Seven infections have been found in the thirty-two cases of this anomaly; three of the proboscis group (*T. vivax*); one of the proboscis and gut group (*T. congolense*) and three of the proboscis, gut and salivary gland group (*T. brucei*). In no case, however, were trypanosomes seen in the degenerated hypopharynx, and the question appears to arise as to whether such flies are capable of transmitting infection.

There also appears to be some doubt as to the passage taken by the trypanosomes in passing from the labial cavity to the hypopharynx.

Some workers maintain that the trypanosomes enter the hypopharynx through a slit in its wall at the proximal end. Authorities, however, are by no means agreed that such an opening exists. If the hypopharynx has no such opening, then it would seem that the trpyanosomes enter at the opening at the extremity of the proboscis.

When the hypopharynx is degenerated as above described, the passage of the trypanosomes of the *T. brucei* group from the gut to the labial cavity, and thence through the tip of the hypopharynx to the salivary glands is easily conceivable. That the trypanosomes of the *T. brucei* group enter the salivary glands through the tip of the hypopharynx seems to be further indicated by the appearance of the long slender proventricular forms at its tip and middle, in normal flies infected with *T. brucei*.

With regard to the transmission of the trypanosomes by tsetses with a degenerated hypopharynx, it is suggested that in the case of *T. brucei* (where this organ is used as a passage only) that such flies could transmit trypanosomes. The normal passage through the hypopharynx would be replaced by the tube formed by the labium and labrum in apposition. Along this both saliva and the mature trypanosomes would pass into the wound when the proboscis punctures the skin.

The infections of the swollen glands were phenomenal, as both glands, throughout their lengths were swarming with trypanosomes, among which were infective forms.

In the case of *T. vivax* and *T. congolense*, where a definite phase of development of the trypanosome takes place in the hypopharynx, it would seem that in flies in which this organ is degenerated, the infection could not mature, and therefore could not be transmitted.

APPENDIX.

Stomoxys—a Possible Transmitter of Nagana.

A severe outbreak of Nagana occurred at the Ntambanana Settlement during the winter of 1932, and complaints were made by the farmers that biting flies were a great pest to the cattle.

Acting on the instructions of the Director of Veterinary Services, the possible rôle played by these biting flies as a transmitting agent of Nagana, was investigated during July, August and September.

The results obtained amount briefly to the following : —

(1) With the aid of the " Harris " trap, *Stomoxys* was shown to be present in enormous numbers at different farms in the Settlement. The species of *Stomoxys* captured were identified as *S. calcitrans*, *S. nigra*, *S. brunnipes* and two species which have not been identified.
    The flies were persistent in their attacks and were a great pest to the cattle.

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(2) Positive cases of Nagana (*T. congolense* and *T. brucei*) were found among the cattle in the Settlement. It is possible that these constituted a focus from which infection was spreading.

(3) *Stomoxys*, dissected soon after feeding on a donkey, whose blood showed very few trypanosomes, were negative in both proboscis and gut.

(4) When there were very numerous trypanosomes in the peripheral blood of the donkey, the flies readily took up the infection.

(5) The longest period that trypanosomes (*T. congolense*) remained active in the gut of *Stomoxys* was twenty hours.

(6) Eight per cent. of *Stomoxys* interrupted while feeding on a donkey, heavily infected with *T. brucei*, were positive in both gut and proboscis.

(7) From this it seems possible that trypanosomes may be conveyed from infected to healthy animals in a mechanical manner by *Stomoxys*, provided that: (*a*) *Stomoxys* is present in large numbers. (*b*) Infected and clean animals are in close contact. (*c*) Very numerous trypanosomes are present in the peripheral blood of the infected animals.

(8) No *Glossinae* were encountered, but no definite search was made for this fly

ACKNOWLEDGMENTS.

REFERENCES.

DUKE, H. L. (1930). On the Susceptibility of the Two Sexes of *G. palpalis* to Infection with *T. gambiense* and *T. rhodesiense*. *Ann. of Trop. Med. and Paras.*, Vol. 24. No. 1.

HARRIS, R. H. T. P. (1932). Some Facts and Figures regarding the Attempted Control of *Glossina pallidipes* in Zululand. *S.A. Jnl. of Sc.*, Vol. 29, pp. 495-507.

NEITZ, W. O. (1931). Blood Parasites of Game in Zululand (Preliminary Report). *17th Rept., Dir. Vet. Serv. and Anim. Indust.*, Union of S.A., pp. 45-60.

WHITNALL, A. B. M. (1932). The Trypanosome Infections of *Glossina pallidipes* in the Umfolosi Game Reserve, Zululand (Preliminary Report). *18th Rept., Dir. Vet. Serv. and Anim. Indust.*, Union of S.A., pp. 21-30.

Section II.

Bacteriology.

Thomsen's Hemagglutination Phenomenon.

Isolation of a "J-Like" Bacillus.

By J. H. MASON, F.R.C.V.S., F.R.S.E., Empire Marketing Board Research Fellow, Onderstepoort.

O. THOMSEN (1927) was the first to report upon a propagatable agent capable of rendering human red corpuscles agglutinable by their homologous (or any other human) serum. Friedenreich (1928, 1930) investigated this phenomenon and showed that the action of bacteria upon the red cells was the cause. Special attention was paid to two germs, designated the " J " and the " M " bacillus, respectively, isolated from somewhat old blood samples.

The following report records the isolation of a germ similar to the " J " bacillus.

In experiments concerned with blood groups in the horse the writer's colleague, Dr. P. J. J. Fourie, noted that the red cells of a certain horse were agglutinated by the homologous serum. This phenomenon occurred after the sedimented cells, in Ringer-Locke solution, had been standing for 48 hours at room temperature; when freshly drawn no hemagglutination had taken place. Further, when a drop of this suspension was added to the red cells of other horses, and 24 to 48 hours allowed to elapse, these cells, in turn, were agglutinated by their homologous sera, controls being negative.

The " J-like " bacillus was isolated by plating a loopful of the suspension on 5 per cent. horse-serum agar at 37° C. for 48 hours. Six different types of colonies were noted and numbered, and from each a small portion was sewn into a red cell suspension obtained under sterile conditions from a horse. The effect of adding homologous serum was noted at 1, 2 and 4 day periods, the cells being all the time at room temperature. After 1 and 2 days one lot of cells was agglutinated and after 4 days a second sample was clumped, the control and the other remaining suspensions of cells being negative. It was decided to more fully investigate the germ responsible for producing the early hemagglutination.

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The " J-LIKE " BACILLUS.

Morphology.—After 24 hours on 5 per cent. horse serum agar, the organism was noted to be a rather small bacillus, showing branching (whether true or false was not investigated) and a large number of V forms. Further incubation (2-3 days) produced much pleomorphism; the majority of the germs were pyriform, but coccoid, bacillary and swollen forms were encountered. It stained readily with methylene and thionin blue, was Gram positive and showed no irregular or beaded staining with Neisser.

Cultivation.—Growth was readily obtained after 24 hours (at 37° C.) on the usual laboratory media, being much sparser and slower at room temperature. On serum-agar after 48 hours, a white, smooth, shining, fairly luxurious growth, resembling a thin streak of paint was obtained, the individual colony being round, raised, white, smooth, and glistening, with an entire edge. In broth and serum broth, a faint to moderate uniform turbidity was noted. Litmus milk was rendered very slightly alkaline and gelatine was not liquefied. None of the following " sugars ", sorbite, inosite, glucose, laevulose, sacharrose, lactose, maltose, dulcite, mannite, galactose, salicin, adonite, inulin, raffinose (1 per cent. in 1 per cent. peptone water) was fermented (as judged by acid and/or gas formation) in 14 days at 37° C.

Young (overnight) broth cultures showed no motility, and 0·5 c.c. of such a culture, injected intraperitoneally into a mouse, produced no ill effects within 7 days.

Effect of Adding the Bacillus to Red Cells.

A loopful or a drop of culture, either broth or agar, was added at different times and as opportunity arose, to cell suspensions of 32 different horses. After standing at room temperature for 24 hours, the homologous serum was added to each sample; in every instance hemagglutination was produced, uninoculated controls being negative.

Effect of Adding Filtrate to Red Cells.

Three 100 c.c. flasks of ordinary broth were sewn with the bacillus and allowed to stand at room temperature. After 1, 3 and 7 days the contents of one bottle were filtered through a Berkefeld candle, tested for sterility and, if sterile, 1·0 c.c. added to 10·0 c.c. quantities of red cell suspension. Such suspensions, after 48 hours at room temperature, were tested, with homologous serum, for hemagglutination. In each instance, hemagglutination was produced, control uninoculated suspensions, standing under the same conditions being negative.

DISCUSSION.

The " J " bacillus as described by Friedenreich is very similar to the germ noted here. The agglutinative effect of both appears to be identical. In their angular type of growth, their biochemical reactions and their lack of action upon such media as gelatin and milk, no difference can be detected. The " J " bacillus would appear to grow less vigorously on agar and to retain Gram's stain less firmly.

than the organism here described. Friedenreich stresses that his bacillus does not grow at 37° C., the optimum temperature being about 20° C. The bacillus obtained from the horse cell suspension was isolated at 37° C. and grown as a routine measure, at this temperature. However, on two occasions this did not hold—(1) a subculture from a month old sealed-off agar slope did not grow at 37° C. but did so at about 23° C. and was then subcultivable at 37° C. and (2) three 100 c.c. flasks of broth inoculated in parallel with those mentioned under the "effect of adding the filtrate to red cells" showed no signs of growth (and no agglutinative effect) after 14 days in the incubator; further sojourn at room temperature produced no growth. It is thus possible that the optimum growth temperature may be determined by a phase of the germ or by some factor unknown.

CONCLUSIONS.

The isolation from a horse red cell suspension of a micro-organism resembling Friedenreich's " J " bacillus, is described. This germ, added to red cells, produced Thomsen's hemagglutination phenomenon.

REFERENCES.

FRIEDENREICH, V. (1928). Investigations into the Thomsen Hemagglutination Phenomena. *Acta Path. Microbiol. Scand.*, Vol. 5, p. 59.

FRIEDENREICH, V. (1930). The Thomsen Hemagglutination Phenomenon. *Contrib. Univ. Inst. for Gen. Path.*, Copenhagen, 1930, Vol. 8, Article 15.

THOMSEN, O. (1927). Ein vermehrungsfahiges Agens als Veränderer des iso-agglutinatorischen Verhaltens der roten Blutkörperchen, eine bisher unbekannte Quelle der Fehlbestimmung. *Zeitschr. fur Imm-Forsch.*, Vol. 52, p. 85.

A Note on the Cultivation of Anaerobes.

By

J. H. MASON, F.R.C.V.S., F.R.S.E., Empire Marketing Board Research Fellow, Onderstepoort.

THE writer (1930) reported that the addition (5-10 per cent.) of a mixture of equal parts of sheep serum and sheep haemolysed red cells (filtered through a Berkefeld candle) to nutrient agar gave better growth results with anaerobes than the addition of serum alone. The results were nearly, and often as good, as those obtained with blood agar and in addition the medium was clear, a decided advantage in the surface isolation of bacteria. The great disadvantage has been the ensuring of sterility of the serum-cell nutrient. Not uncommonly faulty filter candles allowed bacteria to pass and further the fluid had to be stored and samples taken from it whilst in the unpreserved state. Attempts were therefore made to obtain the serum and haemolysed-cells in as sterile a fashion as possible, to destroy any chance bacteria that might have gained access during manipulations, without altering the consistency, appearance or nutritive properties of the mixture and, if possible, to increase the concentration of laked corpuscles in the haemolysed-cell portion.

OBTAINING THE BLOOD.

Sheep were used as donors of blood and in all instances the wool was closely clipped from the jugular region and the animal bled in a relatively draught free, clean room. The bleeding apparatus consisted of a large bore needle, to which was attached a length of rubber tubing, terminating in a piece of narrow glass tubing provided with a cowl. This and all glassware and media used were autoclaved for half an hour at 120° C. prior to use. The effect, on the sterility of the blood, of disinfecting the skin with Tr. iodi, 5 per cent. phenol and 5 per cent. lysol, of touching one small area with 50 per cent. lysol, of a double sterilization (day interval) with 50 per cent. lysol, and of thoroughly cleansing the skin with soap and water prior to disinfection, was noted, by running about 3·0 c.c. of blood directly into broth and Robertson's meat broth and incubating these for 7-14 days at 37° C. The incidence of infection was rather high although it was noted that growth seldom occurred before the 3rd or 4th day, indicating that only a few germs had been introduced. The method finally decided upon and which gave the highest percentage of sterile blood was as follows: The jugular area was clipped and cleansed with alcohol, followed by ether, the needle was inserted into the vein with one thrust and *about 30·0 c.c. of blood allowed to escape;* then the blood was run into sterile tubes or flasks and allowed to clot and a small quantity of blood (to saturation point) laked in distilled water. Whilst this method has

given the best results from a sterility standpoint, the credit for it cannot be entirely allotted to the use of alcohol and ether and the allowing of the first flow of blood to escape. With practice one is able to " hit " the vein at the first thrust, and thus the chances of contaminating the needle point are reduced.

PREPARATION OF THE SERUM-HAEMOLYSED-CELLS MIXTURE.

The serum is allowed to separate and mixed with an equal volume of haemolysed red cells, sterile precautions being adopted.

STERILISATION AND PRESERVATION OF THE MIXTURE.

The effect of adding glycerine 20 per cent., acriflavine 1/5,000 to 1/20,000, formalin (40 per cent. formaldehyde) 0·05 per cent. to 0·2 per cent., hydrogen peroxide 2·5 per cent. of 3 vols., petrol (excess), chloroform (excess) and ether (excess) to the mixture, containing a loopful of a dilute suspension of $S.$ $suipestifer,$ $Bact.$ $coli,$ or a staphyloccus and incubating for 24 hours at 37° C. and then putting up sterility tests, was noted. All except ether were discarded as unsuitable, e.g., acriflavine in non-precipitating concentrations and H_2O_2 did not sterilise, formalin sterilised but was bacteriostatic when the mixture was added to agar and this inoculated with an anaerobe, and petrol and chloroform produced unsightly sediments. Ether answered the purpose satisfactorily. Neither the colour nor the consistency of the mixture was materially altered, excess ether was driven off at 37° C., that remaining in solution being diluted below its effective bacteriostatic range when the mixture was added to agar. To test its bactericidal power a loopful of a dilute suspension of a coliform bacillus, two different staphylococci and a member of the salmonella group was added respectively to tubes (10·0 c.c. amounts) of the mixture and incubated for 24 hours at 37° C. Sterility tests were then put up. The control tubes (mixture plus bacteria without ethér) all showed profuse growth on agar plates and one etherised staphylococcus tube showed a few colonies (indicating a definite although incomplete bactericidal effect) whilst the remaining tubes were sterile. As a routine measure, since this test, an occasional tube of mixture has had added to it a small quantity (usually a loopful) of a dilute suspension of $Bact.$ $coli,$ a staphylococcus or a streptococcus, plus an excess of ether. After incubation for 24 hours at 37° C. a sterility test is conducted. In no instance has growth of the organism been demonstrated; the mixture is either sterile or the number of germs has been greatly reduced as judged by the paucity of growth on plates compared with that from the control unetherised tubes. Further, from a sterility standpoint the method has proved satisfactory in as much as, over a period of 18 months, the incidence of infected agar plates attributable to contaminated mixture has been very low. A few tests, conducted as above, but using anaerobes such as $B.$ $welchii$ and $B.$ $sporogenes,$ showed that ether was unable to kill these germs, even after incubation for 3 days at 37° C., with a poorly sporulating germ like $B.$ $welchii$ there was definite evidence of bactericidal action but with $B.$ $sporogenes,$ which sporulates copiously, this could not be demonstrated.

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Boiled Alkalised Serum and Serum-Haemolysed-Cells.

It is well known that serum or plasma which has been rendered very alkaline by the addition of KOH may be boiled without coagulation occurring. The same applies to serum-haemolysed-cells. Such boiled material when added to media has proved to be growth stimulating to bacteria [Leusden (1932), and Wahby (1932)].

A few experiments were carried out on the growth-enhancing effect of such boiled material on a number of anaerobes. To a series of tubes containing respectively sheep plasma, serum and serum-haemolysed-cells was added from 0·25 per cent. to 1·5 per cent. of N/1 KOH, and the tubes boiled for from ½ to 1 hour. The material was then neutralized with HCl to pH 7·3-7·4, and added in approximately 10 per cent. quantities to nutrient agar and meat broth. These were inoculated with approximately the same quantities of young broth cultures of different anaerobes (*B. welchii*, *B. cochlearius* 2 strains, *B. chauvoei* 2 strains and *B. multifermentans*), and cultivated, under anaerobic conditions, for periods of from one to three days. In brief, the results were disappointing; in every instance the boiled material was less growth-stimulating than the etherised serum-cells mixture (as above) and frequently as good or better growth was obtained in media containing no addition. Table I records one such result; agar containing approximately 10 per cent. of etherised serum-cells, boiled plasma (1 hour, 1·0 per cent. N/1 KOH) boiled serum-cells (as plasma) and with no addition were poured as plates and after drying overnight on the incubator, were streaked with a young *B. welchii* broth culture. They were then cultured for 24 hours in a McIntosh and Fildes' jar.

TABLE I.

Effect of Boiled Plasma, etc., on Growth of B. welchii.

Addition to agar.	No. of colonies.
Boiled plasma	43
Boiled serum-cells	10
Etherised serum-cells	Confluent growth.
Nil	16

In further tests the results varied somewhat, e.g., " boiled serum-cells " was better than " boiled plasma " or " nil " was equal to " boiled plasma " but in all instances the etherised serum-cells gave by far the best results.

Concentration of the Haemolysed-Cell Portion of the Mixture.

The possibility existed that by increasing the concentration of the haemolysed-cell portion of the mixture, better growth results would be obtained. A number of experiments were carried out to determine the haemolysing effect of saponin on oxalated sheep blood. It was found that a dilute solution (1/400) of saponin in distilled water would haemolyse many more red cells than would distilled water alone. A state could be reached where the laked cell fluid was quite mucinous and of a very dark red colour, and further such material mixed with an equal volume of serum and added to agar gave satisfactory growth results. However, comparative tests did not show that such a mixture had any advantage (from a growth-stimulating standpoint) over the routine serum-cell mixture, but on

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the other hand had the definite disadvantage of rendering the medium dark in colour and translucent. It was only with difficulty that bacterial colonies could be clearly seen, viewed from the " back " of the medium. Whilst a point could be reached by the use of a suitable concentration of saponin, where more cells could be laked than in distilled water alone, and the final medium was clear, the fact that no better growth-results were obtained than with the usual method rendered further work in this direction superfluous.

The Use of Etherised Serum-Cells in the Cultivation of Anaerobes.

The writer (1930) has already recorded experiments comparing the growth-stimulating effect of serum and serum-cells from the sheep and horse. It may merely be mentioned here that etherised serum-cell mixture has the same effect as filtered serum-cell mixture. With less delicate anaerobes such as *B. welchii*, *Vibrion septique* and *B. sporogenes*, the growth is as good as that obtained upon blood agar; with *B. chauvoei* the result whilst sometimes not quite so good, is nevertheless, perfectly satisfactory. Further, there is the advantage of being able to keep at hand a supply of sterile material and of having a nearly transparent medium.

Conclusions.

1. A method of obtaining and sterilising a serum-haemolysed-cell-mixture is described.

2. Its growth stimulating effect on anaerobes is noted.

3. Boiled alkalised serum and serum-cells have no growth stimulating action on anaerobes.

References.

LEUSDEN, F. P. (1932). Gekochter Aszites als steriler Nährbodenzusatz. Ein Beitrag über das Verhalten von Eiweisslösungen beim Kochen. *Zentralbl. f. Bakt.*, 1, Orig. Bd. 126, Heft. 5/6, p. 460.

MASON, J. H. (1930). The Cultivation of Anaerobic Organisms. *Vet. Jnl.*, Vol. 86, No. 11, p. 474.

WAHBY, A. M. (1932). Suggested Medium for Growth of *Corynebacterium diphtheriae Jnl. Inf. Dis.*, Vol. 51, No. 3, p. 441.

The Liquefaction of Inspissated Serum by the "Lamb Dysentery Bacillus."

By J. H. MASON, F.R.C.V.S., F.R.S.E., Empire Marketing Board Research Fellow, Onderstepoort.

DALLING (1928) and later Mason, Ross and Dalling (1931) reported that the lamb dysentery bacillus liquefied inspissated horse serum, and emphasized this point when comparing it with classical *B. welchii*. Recently the present writer (1933) examined original and single cell cultures of the lamb dysentery bacillus, *B. paludis* (McEwen) and of *B. welchii*, and found that in no instance was solid serum liquefied. As possible explanations of this apparent anomaly, it was suggested that either the original lamb dysentery culture was contaminated with a proteolytic organism at the time when the first reports were published, or that Löffler's inspissated serum had, in the past, been used instead of solid serum.

While both these possibilities existed and were, at the time, the only ones that occurred as likely explanations, the writer was by no means convinced that they were the true reasons, nor did he feel at ease in suggesting them, for the following two reasons:—

1. The original lamb dysentery culture, upon which most of the work was done, was under constant observation for six years * and was subjected to some manipulation (plating, shaking, etc.), almost daily. With the exception of single cell isolation, every standard method of purifying anaerobes was adopted in the attempt to isolate a proteolytic contaminant. At no time was there any indication that the culture was impure. Further, cultures submitted to and returned from other workers, all expert in anaerobic technique, behaved like the original, both toxico-serologically and culturally.

In addition to the original culture, some 10 other strains were isolated from lambs affected with lamb dysentery. Whilst these were not submitted to the same detailed treatment as the original, still, the platings, shakings, and rapid subcultures that were conducted should have resulted, in all cases, in pure cultures. If the lamb dysentery bacillus had been, at the time, an accepted entity, the resultant cultures would have been accepted as pure.

The main difficulty in the acceptance of the purity of these cultures was the fact that they all rapidly liquefied solid serum. The time elapsing between inoculation and commencement of liquefaction varied, depending on the strain (and no doubt on the amount of inoculum, medium and anaerobiosis), but within one week every strain had to some definite extent caused liquefaction, and usually not more than 48 hours was required to see softening of the medium.

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* At the Wellcome Physiological Research Laboratories, Beckenham.

Whilst the failure to demonstrate a proteolytic contaminant did not rule out the possibility of its presence, there was definite reason to consider that the cultures were pure.

2. That Löffler's serum medium was, on occasion, used instead of coagulated serum, is a possibility, but that it was used on all occasions is extremely unlikely.

The author's attention was again focussed on the subject by statements of Tunnicliff (1933) and of Dalling (personal communication). Tunnicliff, working on a lamb dysentery-like disease in the United States of America, stated that Dalling's lamb dysentery bacillus liquefied solid serum. Dalling, discussing with the writer the toxin-producing power of lamb dysentery and lamb dysentery-like organisms, noted that the toxin produced by his original organism, sealed off since 1922, differed from that produced by a serial subculture of that organism, maintained in the laboratory by short interval subcultures in meat broth. It was such a subculture that was brought to South Africa in 1931 by the author and on which the work already noted (1933) was carried out. Since this investigation was commenced Glenny, Barr, Jones, Dalling and Ross (1933) have published an article in which they state that the lamb dysentery bacillus has, since 1930, undergone a change in its toxin-producing power.[*]

Being given these facts, it occurred to the writer that there was the possibility that, in addition to an alteration in toxin production, the 1930 subculture of the lamb dysentery bacillus had also undergone another change, viz. it had lost the power of liquefying solid serum. The author had at his disposal a number of *B. welchii*-like anaerobes, including, through the courtesy of Dalling, a subculture of the lamb dysentery bacillus " 1922 " and of another recently isolated lamb dysentery strain " U 3 ". The cultures, other than lamb dysentery " 1922 " and " U 3 ", had all been " single celled " many times and no original culture had been retained. All strains were sown from young meat broth cultures on to inspissated horse serum and Löffler's serum medium and incubated in a McIntosh and Fildes' jar at 37° C. No tube (unless liquefaction was definite at an earlier date) was discarded until a month had elapsed.

Löffler's medium was liquefied rapidly by all cultures, being soft and liquid at the bottom of the tube in 5-7 days and nearly completely liquefied in 10-14 days. Charts 1 and 2 give the history of the lamb dysentery " 1922 " and " U 3 " strains respectively on solid serum and Table I of the other organisms on solid serum. It may be stated that when solid serum was liquefied by either lamb dysentery " 1922 " or " U 3 " this was evident in a few days and nearly complete in 10-12 days.

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[*] As this change in the toxin-producing power of the lamb dysentery bacillus will form the subject of a communication by Dalling and his colleagues, the writer is only at liberty to state that such a change has occurred, without indicating the nature of the change.

CHART 1.
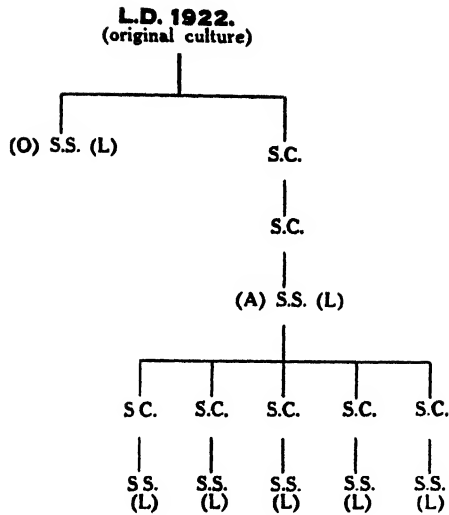
L.D. 1922.
(original culture)

(O) S.S. (L)　　　　　S.C.

S.C.

(A) S.S. (L)

S C.　　S.C.　　S.C.　　S.C.　　S.C.

S S.　　S.S.　　S S.　　S.S.　　S S.
(L)　　(L)　　(L)　　(L)　　(L)

CHART 2.

U 3.
(original culture)

(O) S.S. (L)

S.C.　S.C.　S.C.　S.C.　S.C.　S.C.　S.C.

S.S.　S.S.　S.S.　S.S.　S.S.　S.S.　S.S.
(L)　(L)　(L)　(L)　(L)　(L)　(L)

S.C. = Single cell culture.
S.S. = Solid serum.
L. = Liquefaction.
L.B. = Liquefaction at bottom.
A.O. = See text.

S.C.

S.C.

(A) S.S. (No L)

S.C.　S.C.　S.C.　S.C.　S.C.　S.C.　S.C.

S.S.　S.S.　S S.　S.S.　S S.　S.S.　S.S.
(LB)　(LB)　(LB)　(LB)　(LB)　(LB)　(LB)

35
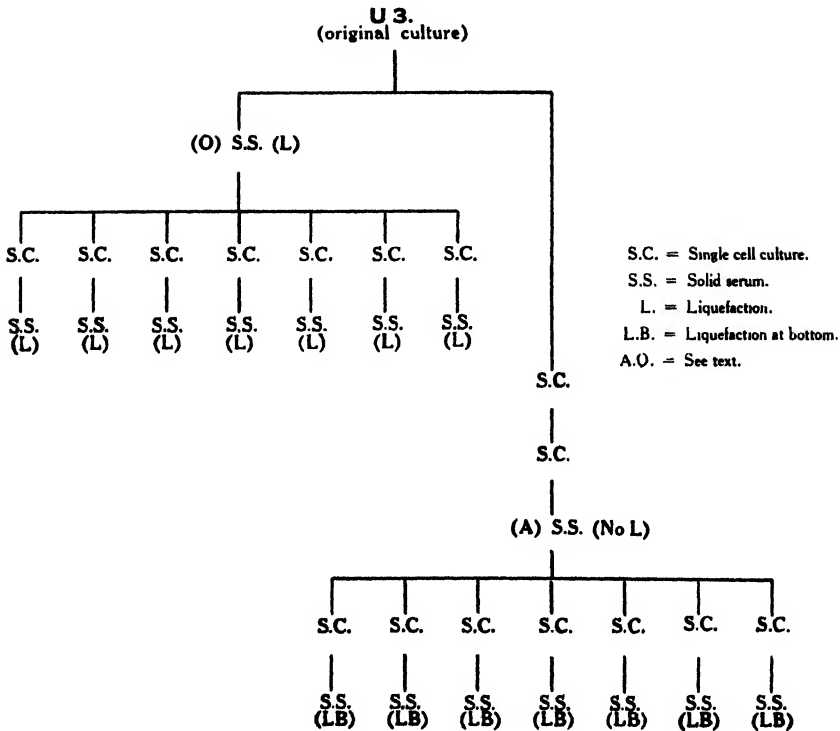
TABLE 1.

Organism.	Effect on Solid Serum (one month).
L.D. 1930 (s.c.)	No effect.
Ovitoxicus (Bennetts) (s.c.)	,, ,,
Paludis (McEwen) (s.c.) ...	,, ,,
Welchii (S.R. 12) (s.c.) ...	Soft at bottom.

TABLE II.   (See Charts 1 and 2.)

Organism.	Type of L.D. Toxin Produced.
L.D. 1922 O	1922
L.D. 1922 A	1930
L.D. 1930 (s.c.)	1930
U 3 O	1922
U 3 A	1922

s.c. = single cell culture.
O and A = See Text and Charts 1 and 2.

DISCUSSION.

Reference to Charts 1 and 2 and to Table I explains the apparent discrepancies in the statements of Dalling and Mason respectively on the serum liquefactive properties of the lamb dysentery bacillus. The orginal and single cell cultures of both lamb dysentery " 1922 " and " U 3 " produce rapid liquefaction, whilst, as previously reported, the 1930 subculture fails to do so. It is remarkable that, quite fortuitously, the first single cell (A) isolated from the " U 3 " strain was non-liquefactive : 6 daughter cells, obtained from it, produced after 3 weeks incubation, a softening and partial liquefaction of the bottom portion of the medium. On the other hand, 7 other single organisms separated from the original (O) serum culture, behaved as did their parent, viz. they produced rapid liquefaction.

Table 2 records the type of toxin produced by the lamb dysentery and " U 3 " strains. It will be noted that the toxin of lamb dysentery " 1922 " (original) and " U 3 " (original and single cell) was of the " 1922 " variety, whilst that of lamb dysentery " 1922 " (single cell) and lamb dysentery " 1930 " (single cell) was of the 1930 type. On each of three separate tests put up with the two last-mentioned strains the same result was obtained.

None of the other three *B. welchii*-like anaerobes produced true liquefaction within one month. *B. welchii*, itself, definitely softened the inspissated serum after 3 weeks' incubation, but this was confined to the bottom portion of the medium and was in no way comparable with the almost complete liquefaction by lamb dysentery " 1922 " in less than one fortnight.

The " U 3 " (single cell) A culture, whilst failing to liquefy solid serum, produced the " 1922 " type of toxin. How often such a variant may be obtained is unknown, but the fact that such a one has been demonstrated shows that the absence of liquefactive power is no proof that a strain cannot produce the " 1922 " type of toxin.

The relative unimportance of liquefactive power in its connection with toxin production is further exemplified by the fact that lamb dysentery (single cell) A brought about rapid liquefaction but yet produced the 1930 type of toxin.

Conclusions.

1. The original statement of Dalling, that the lamb dysentery bacillus rapidly liquefies inspissated horse serum has been confirmed.

2. A serial subculture of Dalling's original strain has lost its liquefactive power.

3. The power of liquefying solid serum (or the lack of this power) should not be applied as a " major " test in classifying a lamb dysentery-like micro-organism.

Acknowledgement.

REFERENCES.

DALLING, T. (1928). Lamb Dysentery. *Nat. Vet. Med. Assoc. Congress Rpt.*

GLENNY, A. T., BARR, M., JONES, M. LL., DALLING, T., AND ROSS, H. E. (1933). Multiple toxins produced by some organisms of the *Cl. welchii* group. *Jnl Path. and Bact.* Vol. 37, No. 1, p. 53.

MASON, J. H. (1933), *B. welchii*, the " Lamb dysentery bacillus " and *B. paludis*. An examination of single-cell cultures. *Vet. Jnl.*, Vol. 89, No. 5, p. 225.

MASON, J. H., ROSS, H. E., AND DALLING, T. (1931). *B. paludis* (McEwen). A comparison with the lamb dysentery bacillus *Jnl. Comp. Path. and Therap.* Vol. 44, p. 258.

TUNNICLIFF, E. A. (1933). A strain of *Cl. welchii* producing fatal dysentery in lambs. *Jnl Infect. Dis.* Vol. 52, No. 3, p. 405.

Section III.

Parasitology.

Descriptions of New Species of Anoplura Parasitic on Antelopes and a Hare.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

SUBORDER MALLOPHAGA.

GENUS BOVICOLA EWING.

Bovicola Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust.*, Un. S. Afr., XVIII. p. 361.

Bovicola pelea nov. sp.

(Figs. 1-3.)

ONE male and several females taken off Vaal Rhebok, *Pelea capreolus* (Bchst.), Naauwpoort, C.P., July 29th, 1932 (coll. Austin Roberts). *Holotype* the male.

Male.—Total length 0·98 mm. Head 0·26 × 0·26 mm. Forehead very slightly emarginated in front. Antennae with the first segment slightly wider than in the female and nearly as long as the second and third segments together. Abdomen widest at the third segment. Tergites i and vii each with a single transverse band and a row of short setae beneath it. Tergites ii-vi each with two narrow, transverse
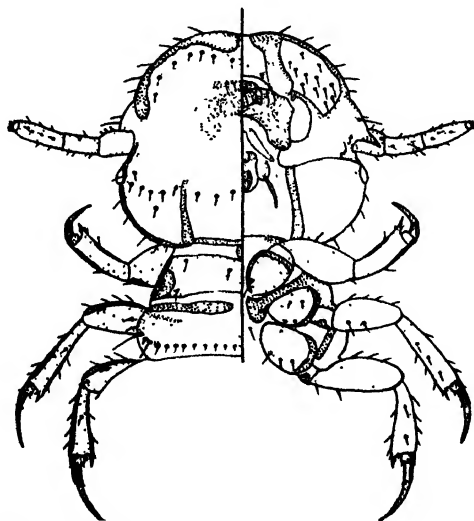
Fig. 1. *Bovicola pelea nov. sp.*, dorsum and venter of head and thorax of ♀
G. A. H. B. del.

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bands, the one being inconspicuous except on tergites ii and vi, and with a transverse row of short setae; on tergite ii the anterior band is emarginated posteriorly. Apical tergite with a small median plate and numerous minute setae. Sternites each with a single broad, transverse band and a row of short setae on the posterior margin. Paratergal plates (=pleurites) only developed on segments i-iv. Male genitalia with the basal plate abruptly constricted near the apex; parameres curved and separated; endomeres elongated, broadest at their bases and pointed at the extremities.
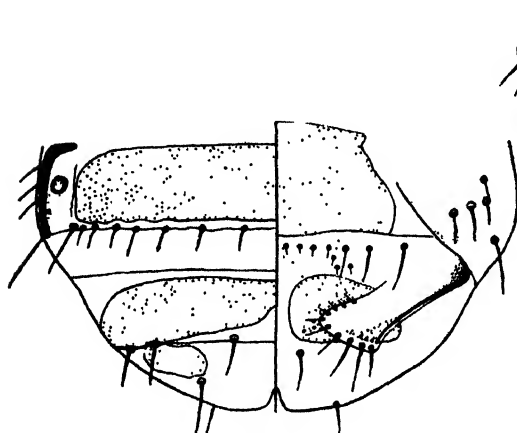
Fig. 2.   Fig. 3.
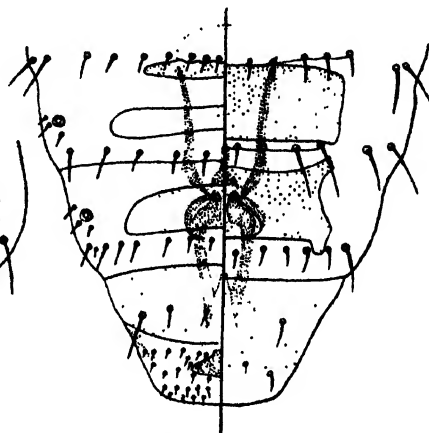
Fig. 2. *Boricola pelea nov. sp.*, apical tergites and sternites of ♀. G. A. H. B. del.

Fig. 3. *Bovicola pelea nov. sp.*, apical tergites and sternites of ♂. G. A. H. B. del.

Female.—Total length 1·26 mm. Head 0·33 × 0·35 mm. Differs from the male in having the first antennal segment shorter and narrower; the paratergal plates are well developed on segments i-vii, and there is only a single transverse band on each tergite, except the last which has a small band on each side, and the band on tergite ii is not emarginated posteriorly.

This species can be distinguished by its small size, being the smallest species of *Boricola* known, also by the male genitalia, gonopophysis of the female and terminal abdominal segments in both sexes. The male resembles *B. painei* (Kellogg and Nakayama) in

having the basal plate constricted near its apex; also in having the transverse band on the second tergite emarginated posteriorly. In *B. painei* the paratergal plates are well developed in the male, and the setae on the abdomen are more numerous in both sexes.

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Bovicola hilli nov. sp.
(Figs. 4-6.)

One male and three females kindly sent by Mr. Laurence Hill, taken off waterbuck, *Kobus ellipsiprymnus* (Ogilby) in the Umfolosi Game Reserve, Zululand. *Holotype* the male in Mr. Hill's collection.
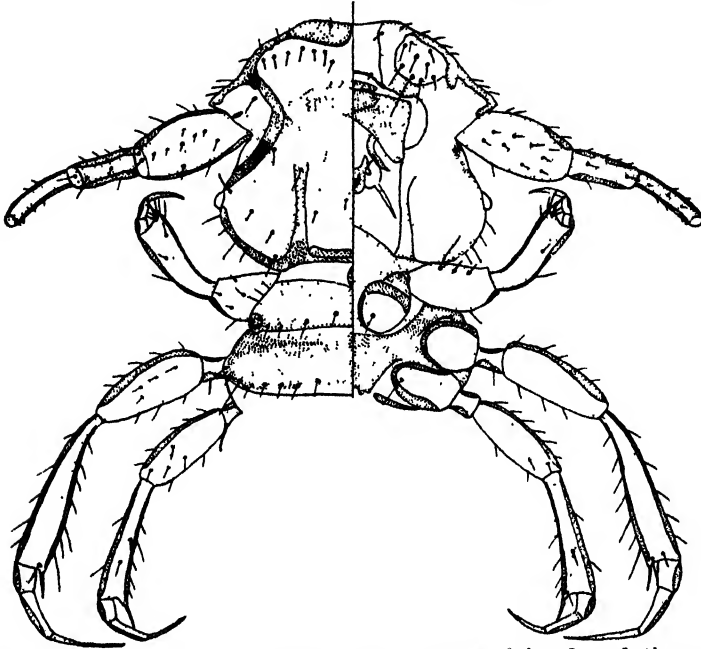
Fig. 4. *Bovicola hilli nov. sp.*, dorsum and venter of head and thorax of ♂. G. A. H. B. del.
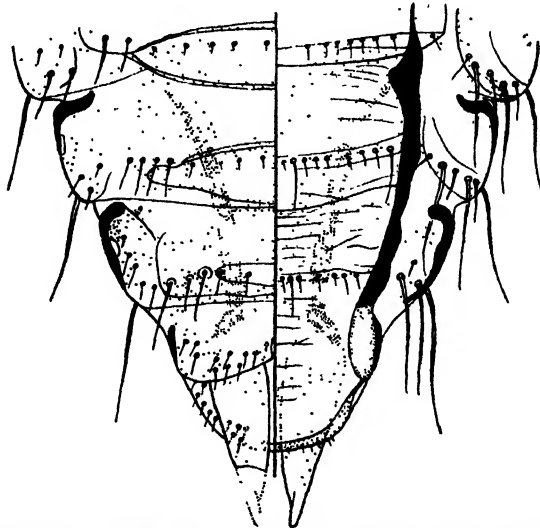
Fig. 5. *Bovicola hilli nov. sp.*, apical tergites and sternites of ♂. G. A. H. B. del.

Male.—Total length 2·11 mm. Head 0·50 × 0·54 mm. Forehead slightly emarginated in front. Antennae with the first segment long and broad, the second the shortest. Tibiae and claws of the mid legs slightly longer than those of the hind legs. Abdomen with crenulated lateral margins, widest at the third segment. Tergites and sternites highly chitinous, each with a transverse row of short setae; a well developed longitudinal plate present on the venter on each side of the male genitalia. Genitalia with basal plate wider at the base than apex; parameres small, apparently fused with the endomeres, which are long and straight, and gradually narrowing from base to apex.

Female.—Total length 2·17 mm. Head 0·50 × 0·54 mm. Differs from the male as follows: The first antennal segment is the shortest, being slightly shorter than the second segment. Tergites and sternites i-v each with a transverse brown band and a row of short setae; the remainder of the segments being almost entirely brown. Gonopophyses shaped like a boot.
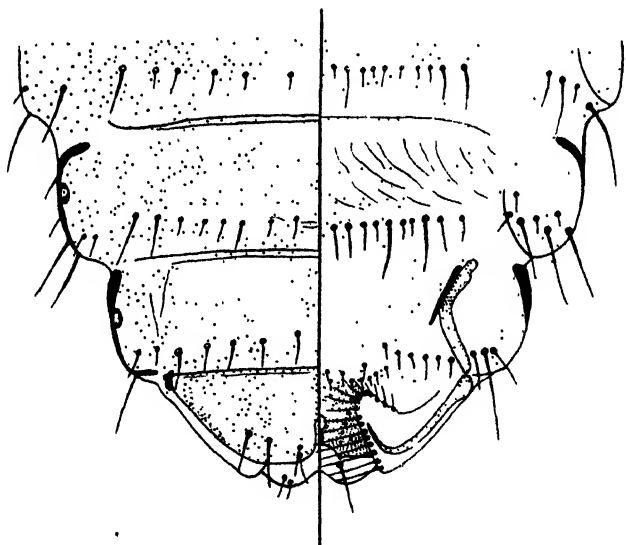
Fig. 6. *Bovicola hilli nov. sp.*, apical tergites and sternites of ♀.
G. A. H. B. del.

This species appears to be closely related to *B. puncta* (Piaget), which was described from a female, obviously a straggler, reported to have been taken off a *Lamprotornis sp.?* (starling) in the Leyden Museum. *B. hilli* is the largest species of *Bovicola* known, and the female can be distinguished from that of *B. puncta* in possessing less setae on the margins of the forehead and temples, and the apical tergites and sternites also appear to be slightly different.

GENUS TRICHOLIPEURUS BEDFORD.

Tricholipeurus Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim.*

Indust., Un. S. Afr., XVIII, p. 363.

In 1929 I described a new species, *Tricholipeurus aepycerus*, reported to have been taken off an impala, *Aepyceros melampus*, on the Kunene River, South-West Africa. Recently I received several specimens, which prove to be new, collected by Dr. Thomas and Mr. Neitz off the same host in the Kruger National Park, Transvaal. On enquiring from Mr. Austin Roberts whether the western impala was the same species as the eastern form, he informed me that *A. melampus* had a wide range in South Africa and extended from the
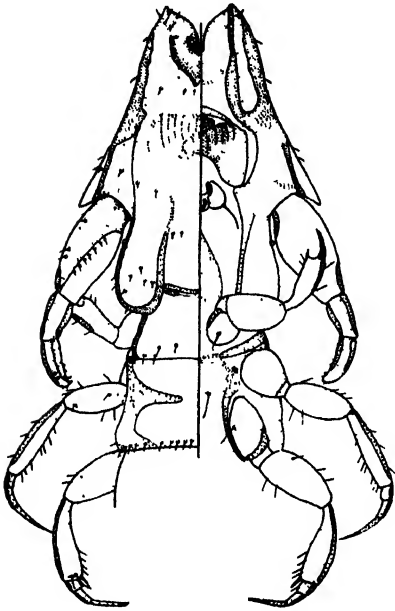
Fig. 7.   Fig. 8.

Fig. 7. *Tricholipeurus elongatus nov. sp.*, dorsum and venter of head and thorax of ♂.
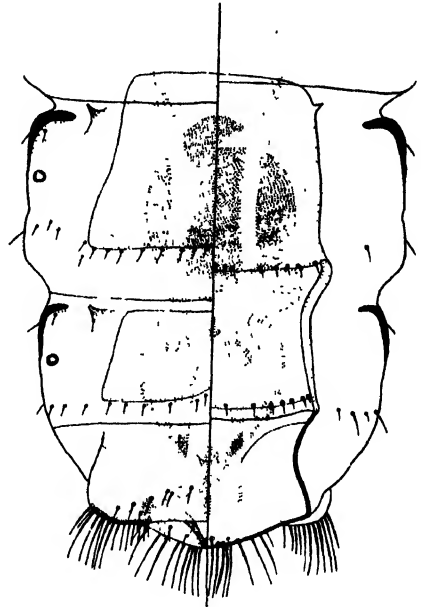
G. A. H. B. del.

Fig. 8. *Tricholipeurus elongatus nov. sp.*, apical tergites and sternites of ♂.
G. A. H. B. del.

east to the west; also that a second species—the Angola impala, *Aepyceros petersi* Boch, occurred in the west, and Captain Shortridge had informed him that he had found this species as far south as the Kunene River. From this it would appear that *T. aepycerus* was taken off *A. petersi* and not *A. melampus*, and that the latter buck is the true host of the new species described below.

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Tricholipeurus elongatus nov. sp.

(Figs. 7-9.)

Male.—Total length 2·76 mm.   Head 0·63 × 0·36 mm.   Forehead deeply emarginated in front, with a transverse row of only six minute setae behind the clypeal plates.   Antennae with the first segment broad, slightly longer than the second and third together; third segment with numerous very minute spines on the inner margin. Legs with the mid tibiae narrower than the fore and hind tibiae, and the claws of the mid legs are slightly longer than those of the hind legs.   Abdomen elongated and narrow with crenulated lateral margins.   Tergites and sternites each with a largish median plate,
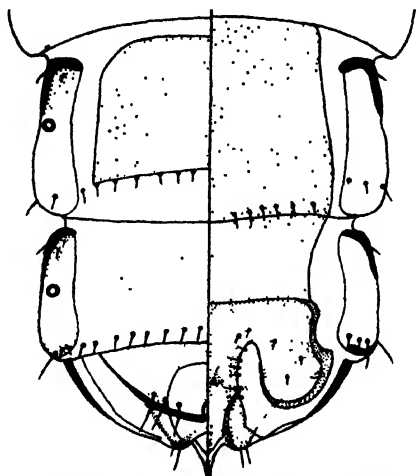
Fig. 9. *Tricholipeurus elongatus nov. sp.*, apical tergites and sternites of ♀. G. A. H. B. del.
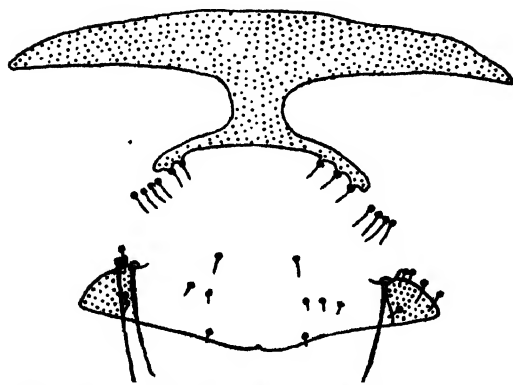
Fig. 10. *Haemodipsus africanus nov. sp.*, genital region of ♀. G. A. H. B. del.

also a transverse row of short setae, except on sternite i; tergite i with a narrow marginal band; tergites ii to vi each with a comma-shaped plate in front of each spiracle, and close to these and nearer the middle there is a small, forked chitinous marking. Genitalia with the parameres joined, forming a pseudopenis which is asymmetrical at the junction of the arms; endomeres long and straight, wider at their bases than at their apices, above them there is a small median plate.

Female.—Total length 2·8 mm. Head 0·63 × 0·36 mm. Differs from the male in having the first antennal segment shorter and narrower, and the median bands on sternites v to viii are fused. Gonopophyses shaped like a boot.
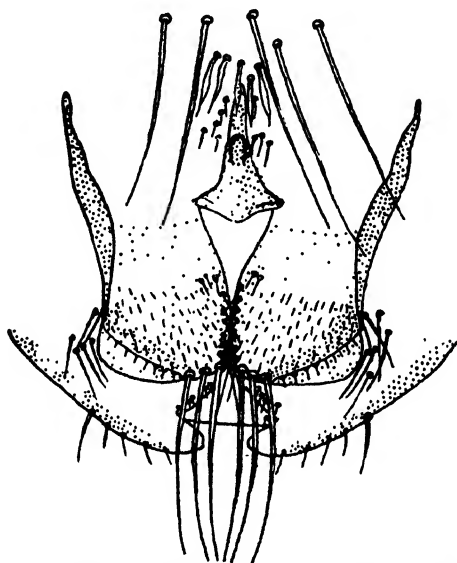
Fig. 11. *Linognathus lewisi* nov. *sp.*, genital region of ♀.
G. A. H. B. del.

Holotype a male.

T. elongatus is the longest species known. It resembles both *T. aepycerus* Bedford and *T. lerouxi* Bedford in having the forehead deeply emarginated in front. From both these and the other known species it can be distinguished by having the temples projecting backwards on to the prothorax, also by the apical tergites and sternites and male genitalia.

SUBORDER SIPHUNCULATA.

GENUS HAEMODIPSUS ENDERLEIN.

Haemodipsus Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 407.

Haemodipsus Ferris, 1932, *Contrib. Toward a Mon. Suck. Lice. Stanford Univers. Pub. Univers. Series. Biol. Series*, II, No. 5, p. 59.

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Haemodipsus africanus nov. sp.
(Fig. 10.)

A single female taken off *Lepus zuluensis* Thos. and Schw., Jericho, Transvaal.

Female.—Total length 1·9 mm. Resembles *H. lyriocephalus* (Burm.) of which Ferris (1932) has given an excellent figure, in the shape of the head, thorax and abdomen, also in the chaetotaxy. From this species it can be distinguished in possessing paratergal plates (=pleurites) on the third to sixth segments as in *H. ventricosus* (Denny) and *H. setoni* Ewing. They are very small, merely forming a slight tooth as in *H. ventricosus*. Sternal plate on the thorax transverse and narrow, similar to that of *H. ventricosus*. The genital plate (Fig. 10) is slightly more developed than in the other species, and the gonopophyses even less developed, being merely indicated by a row of short setae on each side below the genital plate.

GENUS LINOGNATHUS ENDERLEIN.

Linognathus Bedford, 1932, *Rep. Dir. Vet. Serr. and Anim. Indust. Un. S. Afr.*, XVIII, p. 408.

Linognathus, Ferris, 1932, *Contrib. Toward a Mon. Suck. Lice. Stanford Univers. Pub. Univers. Series. Biol. Series*, II, No. 5, p. 66.

Linognathus lewisi nov. sp.
(Fig. 11.)

Three females taken off *Gazella thomsoni*, Gunth., Naivasha, Kenya Colony (coll. E. A. Lewis).

This species belongs to a small group of forms which includes *L. pithodes* Cummings, *L. bedfordi* Ferris and *L. spicatus* Ferris, all of which Ferris (1932) has given excellent figures. They are all parasitic on antelopes and very closely related to each other. They may be distinguished in having a short, broad head, broad thorax and abdomen, and numerous lanceolate setae on the dorsum and venter of the abdomen. Both *L. bedfordi* and *L. spicatus* are only found on the bare parts of their hosts, such as the thighs, axillae and eyelids, and it is quite possible that the other species belonging to this group have similar habits.

L. lewisi resembles both *L. pithodes* and *L. bedfordi* in having the claws of the anterior legs more slender than the others, whereas in *L. spicatus* they are stouter and almost the same size as those of the mid and hind legs.

Female.—Total length 1·6 mm. Head and thorax as in the other species included in the group. Abdomen with the lanceolate setae slightly more numerous than in *L. pithodes* and *L. bedfordi*. Genital plate widened at the apex, whereas it is linear in *L. bedfordi* and absent in the other two species; above the plate there are five long setae. Gonopophyses touching in the middle, with three long setae at their rounded extremity; at their apices there is a small plate on each side, these being absent in the other species.

South African Ticks.

PART I.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

IT is 25 years ago since Nuttall and Warburton published Part I of their Monograph of the Ixodoidea, and Howard his paper on South African ticks in the Annals of the Transvaal Museum. As both these works are now out of date, the former has never been completed, and the latter is now out of print, it was considered advisable, in view of the importance of ticks, to prepare the present work. Owing to the cost of publishing this work it will appear in about four parts in the Onderstepoort Journal of Veterinary Science and Animal Industry. Sufficient reprints will be printed so that the work, when complete, can be published in book form. Part I deals mainly with the species of Argasidae. A complete list of references will be given in the final part. A number of them will be found in " A Synoptic Check-list and Host-list of the Ectoparasites found in South African Mammalia, Aves and Reptilia. Second Edition ", published in Ann. Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr., 1932, XVIII, pp. 515-517. Only a list of works referring to the transmission of diseases by species of Argasidae will be found at the end of this paper.

It is hardly necessary to emphasize the importance of ticks as it is well known that they play an important rôle in the transmission of a number of diseases to man and domestic animals. Apart from this they also do a considerable amount of harm to animals when numerous by sucking blood and setting up irritation, which cause their hosts to lose their appetites and fall off in condition, and may even cause the death of their host without transmitting a disease. Theiler (1911) recorded a case in which a horse, badly infected with blue ticks (*Boophilus decoloratus* Koch) died from acute anaemia as a result of the tick infestation. No less than 14 lb. of blue ticks were collected from this animal in three days, and this amount only represented about half the number of ticks which had gorged on this animal.

Fig. 1. Horse badly infected with *Boophilus decoloratus* (Koch). Engorged female ticks plainly visible.

[*Photo T. Meyer.*

The spinose ear tick (*Argas megnini* Dugès), which was introduced into this country from America, has often been known to kill sheep, goats and calves by the irritation set up when feeding in the ears (it being usually only found in the ears of its host).

The bites of some species of *Hyalomma* and *Amblyomma* sometimes cause sores which may lead to sloughing.

Fig. 2. Neck of same horse.

[*Photo T. Meyer.*

As our knowledge concerning the hosts of various species of ticks is by no means complete, and as certain species may feed only on certain kinds of animals in their immature stages, and on other animals in their adult stages, it is important that we should be able to identify all the stages of a species of tick, especially as it is frequently inconvenient, if not impossible, to breed the larvae and nymphs collected from animals or birds shot in the veld to their adult stages. The only ticks in which I have up to the present been unsuccessful in detecting specific characters have been the larvae of *Amblyomma*.

EXTERNAL ANATOMY.

The body is usally oval in shape and flattened dorso-ventrally in unfed specimens, becoming swollen after feeding, except in the males of *Ixodidae*. The *capitulum* (rostrum or false head) is situated in front in the *Ixodidae* and lies in a *camerostome* or hollow on the venter in the *Argasidae*. It consists of the following parts:—
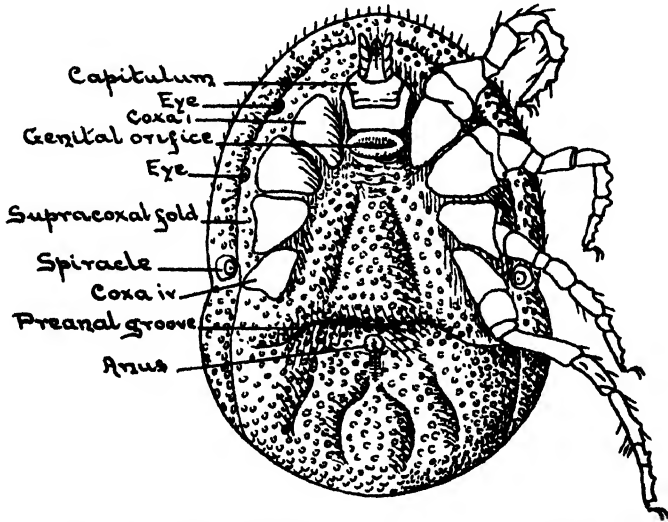
Fig. 3. *Argas savignyi* Aud., venter of female.
C. G. Walker del.

The *basis capituli* which is the basal portion articulated with the body. In the Ixodidae a *dorsal ridge* with backward projecting edge is often present. It may have protruding angles termed *cornua*. A *ventral ridge* may likewise be present, also a protruding retrograde process at the lateral angles of the ventral ridge termed the *auricula*. The females of Ixodidae have a pair of depressions on the dorsum known as *porose areas*, the space between them being called the *interval*.

The *hypostome*, which arises from the basis capituli, projects forward in front and bears backward-projecting teeth (except in the adults of *A. megnini*), which are usually arranged in longitudinal

files. The number of files varies in different species, and 3/3 indicates that there are three files on each half of the hypostome. The hypostome may be rounded, pointed or emarginated at its apex, and if a number of minute teeth are present at its tip, it is described as having a *corona*.

The *chelicerae* or mandibles are paired organs lying above the hypostome. Situated at the extremity of each chelicera is a *digit* or cutting organ consisting of an *internal article* which has a *dorsal process* projecting outwards near the apex, and an *external article* articulating with the internal article on its inner side.
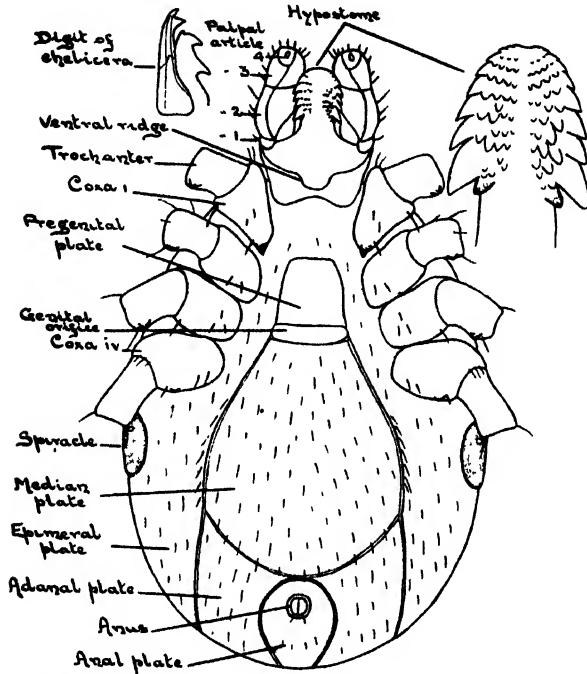
Fig. 4. *Ixodes pilosus* Koch, venter of male.

G. A. H. B. del.

The *palpi* are situated one on each side of the hypostome. In the Argasidae they are always four segmented, flexible, and have the fourth segment terminal. In the Ixodidae they are usually four-segmented, but some of the segments may be fused, especially the second and third segments. The first three segments are usually grooved on their inner margins. In all species, except *N. namaqua*, the segments are rigid, and the fourth segment is very small and lies in a cup-like hollow on the venter of the third segment.

Body.—In the Ixodidae there is a chitinous shield or *scutum* on the dorsum. In the adult males it often covers the whole of the upper surface, but may, as in all *Ixodes*, be surrounded by a raised fold of the body, termed the *marginal fold*. In all the other stages the scutum forms a small oval or round plate behind the capitulum. Some males possess a ridge or punctations on the scutum outlining in shape and position the female scutum, this being termed the *pseudoscutum*.

In a number of species the scutum is of a uniform colour, but many have well-marked colour designs and spots on the scutum, when it is spoken of as *ornate*. Small circular depressions, termed *punctations*, are usually present on the surface. The antero-lateral angles of the scutum may be prolonged into well-marked shoulders, the *scapulae*. Extending backwards from the inner angles of the scapulae are the *cervical grooves*, and running along the sides of the scutum are the
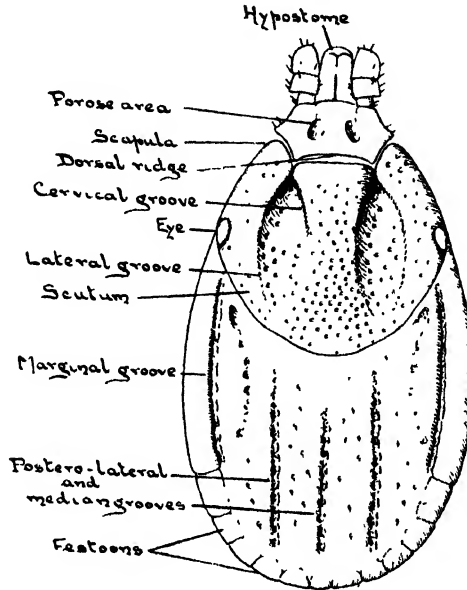
Fig. 5. *Rhipicephalus appendiculatus* Neu., dorsum of female.
C. G. Walker del.

lateral grooves, which in the male may extend backwards and include one or more festoons. The female also has *marginal grooves* running along the sides of the body, these corresponding to the lateral grooves in the male. Depressions, often ill-defined, known as the *median* and *postero-lateral depressions*, *furrows* or *grooves*, may be present towards the posterior margins in the males and females of certain species. Two small spots, termed the *foveae*, may also often be seen near the middle of the scutum in the males, and posterior to the scutum in the females. Uniform rectangular areas, known as *festoons*, are frequently present on the posterior margins of Ixodidae. They are visible both dorsally and ventrally, but more distinct in unfed than gorged females. They sometimes have chitinous plates on their ventral surfaces.

Venter.—Situated on the under surface of the body is the *genital orifice*, termed the *vulva* in the female, which lies in the median line behind the basis capituli. It will be seen to be a transverse slit, and is wider in the females of Argasidae than in the males. Arising in front of the orifice, which it covers, is a chitinous flap called the *apron*. Extending from the sides of the genital orifice are two well-marked grooves, the *genital grooves*, which run backwards between the coxae and then laterally towards the posterior margin of the body.
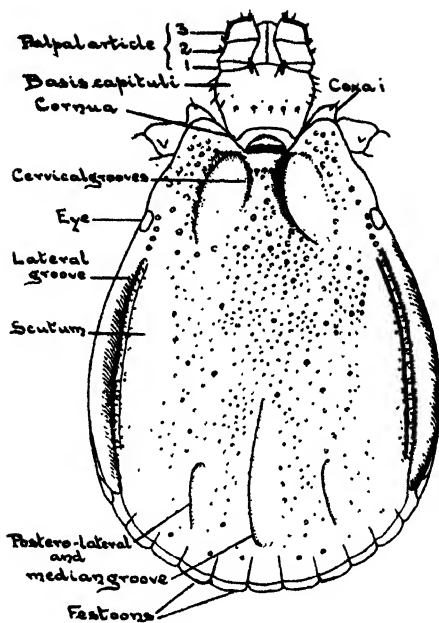
Fig. 6. *Rhipicephalus appendiculatus* Neu., dorsum of male.
C. G. Walker del.
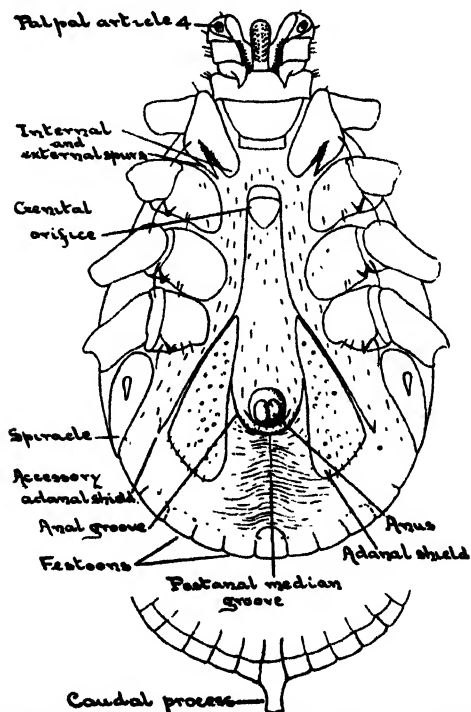
Fig. 7. *Rhipicephalus appendiculatus* Neu., venter of male.
C. G. Walker del.

The *anus* lies in the median line posterior to the hind coxae, and behind this is a median longitudinal groove, the postanal groove. In the Ixodidae there is usually an *anal groove* present, which either curves round the anus in front or behind. In the males of *Ixodes* there are several flat chitinous plates present; these include one *pregenital*, one *median*, one *anal*, two *adanal* and two *epimeral plates* (see Fig. 4). The males of *Rhipicephalus*, *Boophilus* and *Hyalomma* possess a pair of salient chitinous structures, known as the *adanal shields*, situated one on each side of the anus, and external to these a pair of *accessory adanal shields* are frequently present (see Fig. 7). In the Argasidae there is a well-developed fold, the *supra-coxal* fold, extending on each side from the lateral margins of the camerostome along the outer margins of the coxae, and on the inner margins of the coxae a pair of *coxal folds* are frequently present. The camerostome may project forwards in front of the capitulum, forming a *hood*.

Spiracles.—In the Ixodidae the spiracles are situated ventro-laterally, one on each side behind coxae iv. Only in one species, *N. namaqua*, has it not been possible to demonstrate their presence. They are circular, oval or comma-shaped, and may have a marginal frame of chitin, which is always incomplete postero-ventrally in the comma-shaped forms. Situated at or near the centre of each spiracle is a round or elongate structure termed the *macula*. The spiracles of *Haemaphysalis cinnabarina punctata* Can. and Fan., have been studied by Nuttall, Cooper and Robinson (*Parasit.*, 1908, I, iv, pp. 347-351, pl. 22, 23). In the Argasidae they are smaller and situated more anteriorly, usually between legs iii and iv.

Eyes may be either absent or present. If present, there are two pairs in the Argasidae and one pair in the Ixodidae. In the former they are situated on the supra-coxal folds, the first pair in a line with coxae i and the second pair between coxae iii and iv; and in the latter they are situated on the lateral margins of the scutum.

Legs.—The legs are six-jointed, the first joint being the coxa, which is immovable, followed by the *trochanter*, *femur*, *tibia*, *protarsus* and *tarsus*, and in addition to these false articulations may be present. At the end of the tarsus there is a stalk to which are attached two well-developed *claws*. On the ventral surface of the claws there is a disc-like expansion, the *pulvillum*, which is well-developed in the Ixodidae, but absent or rudimentary in the Argasidae. Situated on the dorsum of the first pair of tarsi is a sense organ known as *Haller's organ*; it consists of several cup-shaped pores containing sensory setae and dermal cells. It has been described by Nuttall, Cooper and Robinson (*Parasit.*, 1908, I, iii, pp. 238-242, t.f. 1, pl. 18). In the Argasidae the tarsi frequently have *dorsal pro-truberances*, and in the Ixodidae they either taper or are humped, and are often armed with one or two *ventral spurs*.

INTERNAL ANATOMY.

The internal anatomy of *Argas persicus* (Oken) has been fully described by Robinson and Davidson (1913-14: *Parasit.*, VI, i, pp. 20-48, t.f. 1-2, pl. 1-6; *ibid.*, VI, iii, pp. 217-256, t.f. 1-8, pl. 14-17; *ibid*. VI, iv, pp. 383-424, t.f. 1-8, pl. 25-28). Patton and Cragg, A Textbook of Medical Entomology, 1913, pp. 651-674, pl. 83-86) have also described the internal anatomy of *A. persicus* and other species.

CLASSIFICATION.

Ticks belong to the order ACARINA, which also includes the mites. They are usually much larger than mites, except their larvae, and can be distinguished from them by possessing the following combined characters :—

(i) A pair of spiracles situated laterally, either behind the posterior pair of legs or between the third and fourth pairs. Only in one species, *Nuttalliella namaqua*, has it not been possible to detect spiracles.

(ii) The tracheae opening through a chitinous plate.

(iii) Having a movable false head, or capitulum, of a special structure.

(iv) The hypostome is large and provided with backward-projecting teeth. Only in the adults of *A. megnini* is the hypostome unarmed, and in the female of *N. namaqua* the teeth are very rudimentary.

(v) Possessing a sense organ, known as Haller's organ, on tarsus i.

The ticks are included in the superfamily IXODOIDEA, which is sub-divided into two families as follows :—

Family ARGASIDAE.—Integument of body more or less leathery, without a hard shield (scutum). Sexual dimorphism slight, the males only being distinguishable from the females by the shape of the sexual opening. Head situated on the anterior portion of the ventral surface, and not projecting beyond the anterior margin of the body, except in the larvae, and also sometimes in the nymphs of *A. megnini*. Eyes usually absent; when present, four in number and situated laterally on the supra-coxal folds. Pulvillus absent or rudimentary.

Family IXODIDAE.—Scutum present on the dorsal surface of the body, forming a small round or oval plate behind the capitulum in the females, nymphs and larvae, and covering or practically covering the entire upper surface in the males. Head situated on the anterior margin, and always plainly visible when viewed from above. Eyes absent or present; when present, two in number and situated on the lateral margin of the scutum. Pulvillus always present.

In addition to the above, there are other characters by which these two families can usually be distinguished, but they do not hold good for *Nuttalliella namaqua* Bedford, which is included in the family Ixodidae. This species is particularly interesting as it appears to be the missing link in the elovutionary chain between the Argasidae and Ixodidae, and seems to indicate that the Ixodidae may have originated in Africa. It resembles the Argasidae in possessing a leathery integument, and has a scutum as in the Ixodidae, but instead of being highly chitinous as in that family, it more closely resembles the rest of the integument of the body. The joints of the palpi are very flexible as in the Argasidae, not ridged as in the Ixodidae; the fourth segment is terminal as in the Argasidae, whereas in the Ixodidae it is situated ventrally at the distal end of the third segment, and is reduced, forming a tactile papilla; the second segment is grooved. The Argasidae possess ungrooved palpi, whereas in the Ixodidae the second and third segments are grooved on their inner margins, except in the male of the exotic species, *Ixodes putus* (Pickard-Cambridge), which is parasitic upon birds.

In the Ixodidae the spiracles are generally large and situated well behind coxae iv, whereas in the Argasidae they are small and situated more anteriorly. In *N. namaqua* these organs could not be located, although they should exist. Apart from external characters, the species of Argasidae also differ from those of the Ixodidae in their biology.

THE LIFE CYCLES OF TICKS.

All ticks are blood-sucking parasites of mammals, birds, reptiles and amphibia, but no species has been recorded from the last named hosts in Africa. Some species are only parasitic on hosts that are closely related to each other, whereas others are found on a number of different hosts that are in no way related to one another. Moreover, the immature stages of one or two species, such as *I. rubicundus* and *H. aegyptium*, are rarely, if ever, found on the same hosts as the adults.

ARGASIDAE.—The females feed several times, and after each meal crawl to some sheltered spot where they lay a small batch of eggs. The total number of eggs laid rarely exceeds a thousand. The egg stage usually lasts about one to three weeks, and a few days after hatching the young larvae crawl about in search of a host, except those of *A. moubata* and *A. savignyi*, which are unable to feed, and on getting on to a suitable host immediately commence to suck blood. They remain feeding on their host for a few days, and then drop off and seek shelter, where they moult into nymphs a few days later. Both the nymphs and adults live most of their time in sheltered places and only seek a host at intervals to feed. They invariably only feed at night, and are rapid feeders, taking from 30 minutes or less to an hour or two to feed. In *A. persicus* and *A. respertilionis* there are two nymphal stages, and in *A. moubata* and *A. savignyi* the male nymphs undergo 3 to 5 ecdyses and the female nymphs from 3 or 4 to 6 or 7 ecdyses before moulting into adults. Pairing takes

place off the host, either before or after feeding. The life cycle of *A. megnini* is unique in that the adults do not feed; also, the larvae after feeding moult into nymphs on their hosts, and the nymphs remain attached to their hosts for one to twelve weeks or longer.

IXODIDAE.—The females remain on their hosts until they are fully gorged. They then drop off and hide in grass or under stones, and in a few days commence to lay eggs. Laying continues until the bodies of the females are flat and empty, then they die. The number of eggs laid by a single female varies according to the species. *B. decoloratus* lays from 1,000 to 2,500; *R. appendiculatus* from 3,000 to 5,700; *H. aegyptium* from 10,000 to 15,500 and *A. hebraeum* up to 18,500. The egg stage lasts about 3 to 6 weeks or longer, the period depending upon the species and climatic conditions. The larvae are ready to feed a few days after escaping from the eggs, and then crawl up the stems of grasses or other plants where they frequently accumulate in masses, and wait for a host to pass by. Should they be so unfortunate as to not meet with a host, which many, in fact the majority, that hatch in the course of a year undoubtedly do, they must, of course, eventually die of starvation. On the other hand, should a suitable host pass within their reach they promptly cling to it by means of their legs, and having selected a suitable spot, insert their mouth-parts into the flesh and commence to feed. From now on various species differ in their habits, and may be classified into three groups as follows:—

One-host Ticks.—Ticks which moult from the larval stage into the nymphal stage, and from the nymphal stage into the adult stage on the same host. To this group belongs the species of *Boophilus* and *Margaropus winthemi.*

Two-host Ticks.—The larvae of these ticks moult into nymphs on their hosts, but the nymphs drop off after feeding. After remaining on the ground for about three weeks they moult into adults, which crawl up vegetation and wait for another host. To this group belongs *Rhipicephalus evertsi* and *Hyalomma aegyptium.* The later is also sometimes a three-host tick.

Three-host Ticks.—The larvae of these ticks, having found a suitable host and gorged, drop off and, after remaining on the ground for some time, moult into nymphs which, in turn, have to find another host. Having done so, they gorge and then drop off, and, after a lapse of time, moult into adults, which again have to seek another host. To this group belongs the majority of Ixodidae.

Adults belonging to all the above groups usually pair on their hosts, and the males remain on their hosts much longer than the females, and never, except in exceptional circumstances perhaps, drop off their host to find another one, or get on to another animal that may happen to come in contact with its host. The males of a few exotic species, chiefly *Ixodes,* are known not to feed. These, after emerging from the nymphal stage, lie in wait for the females to drop off gorged from their host in its burrow or nest. It is possible that the males of the three following South African species do not feed: *Ixodes daveyi* Nutt., *I. nairobiensis* Nutt., and *I. simplex* Neu.

MODE OF FEEDING.

The method of feeding is similar in all ticks. A tick penetrates the tissue of its host by means of its paired chelicerae, which can be moved backwards or forwards. Situated at the extremity of each chelicera is a digit or cutting organ, which is provided with backward-projecting teeth and can be moved laterally by means of two tendons. When a wound has been made the hypostome is inserted into it. This is provided with backward-projecting teeth which serve to anchor the tick to its host. When the chelicerae and hypostome have penetrated sufficiently and the digits have cut the smaller blood vessels, the tick proceeds to imbibe blood, this being sucked in by means of a pumping organ, the pharynx, which is situated in the capitulum. Entering the pharynx the blood passes through the oesophagus into the intestinal caeca, and then the tick proceeds to swell. At first the blood is imbibed slowly, the maximum increase in the engorgement of ticks belonging to the Ixodidae usually taking place during the last twenty-four hours before they abandon their hosts.

Whilst feeding, not only is saliva injected by the tick from its salivary glands into the wound, but a considerable quantity of secretion may be expelled from large pores situated between the first and second pairs of coxae. Both the secretions from the salivary and coxal glands have been shown by Nuttall to contain an anticoagulin, which obviously prevents the blood from coagulating and promotes its flow. There is reason to suppose that the secretions may at times exert a toxic action. During feeding ticks also frequently void excreta.

Family *ARGASIDAE* Canestrini.

This family has in the past comprised two genera, *Argas* and *Ornithodoros*, the former only distinguishable from the latter in having the margin of the body differing in structure from the rest of the integument. Recently, in 1932, I sank *Ornithodoros* as a synonym of *Argas*, and pointed out that in 1908 Nuttall, Warburton, Cooper and Robinson stated that they were by no means sure that the family *Argasidae* contained more than one genus, *Argas*, and that since then one or two species have been described which support their view. I also drew attention to the fact that in *Ornithodoros* the integument varies in different species, and in some, such as *O. perengueyi*, it more closely resembles the integument of certain species of *Argas* than of other species of *Ornithodoros*, such as *moubata*, etc. My chief reason, however, for sinking *Ornithodoros* is because in *O. megnini* and several other species included in the genus *Ornithodoros*, the whole of the integument differs from that of *O. savignyi*, the type of genus, and if the whole of the integument is of no generic significance, then it is unreasonable to consider a portion of the integument (the margin) as being of generic importance, especially as the differences in the latter are no greater than the differences in the former. Moreover, in *O. megnini* the integument of the nymph differs considerably from that of the adults. To place all the species in two genera is, therefore, as far as I can see, entirely out of the question. Either the species must be included in a single genus, *Argas*, or else several genera will have to be established. After going

carefully into the matter, and taking not only the external anatomy but also the biology of the ticks into consideration, I have come to the conclusion that the former procedure is the only reasonable one to adopt, at any rate at the present time.

Genus ARGAS Latreille.

Argas Latreille, 1796, *Précis Caract. Ins.*, p. 178.
Carios Latreille, 1796, *ibid.*, p. 177.
Rhynchoprion Hermann, 1804, *Mém. aptérolog.*, XII, p. 69.
Ornithodoros Koch, 1844, *Arch. f. Naturg.*, X, 1, p. 219.
Ornithodorus Karsch, 1878, *Zeitschr. f. ges. Naturwiss.* (3) III, p. 321.
Alectorobius Pocock, 1907, *Allbutt's Syst. Med.*, V, ii, (2), p. 189.
Otobius Banks, 1912, *Proc. Ent. Soc. Wash.*, XIV, p. 99.
Argas Nutt., Warb., Cooper and Robinson, 1908, *Ticks: Mon Irod.*, i, p. 39.
Ornithodoros Nutt., Warb., Cooper and Robinson, 1908, *ibid.*, i, p. 39.
Genotype: Argas reflexus (Fabricius).

This genus is widely distributed and comprises about thirty-two species, of which ten have been recorded from South Africa.

Key to the South African Adults and Nymphs.

1. Integument not mammillated; eyes absent 2
   Integument mammillated or granular, usually without discs; eyes present or absent 7
2. Body of adults and 2nd stage nymph wider than long with anterior margin pointed; of 1st stage nymph circular *A. vespertilionis* (Ltr.) p. 61
   Body of adults and nymphs oblong 3
3. Integument of adults with numerous small pits, of nymph with numerous small spines in front and setae behind; margins similar to rest of body ... *A. megnini* Dugès, p. 77
   Integument without spines or small pits, but symmetrically arranged discs (largish depressions) present 4
4. Anterior margin of body rounded 5
   Anterior margin of body sub-conical 6
5. Margin of body formed of quadrangular plates *A. persicus* (Oken) p. 65
   Margin of body formed of a series of irregular wrinkles *A. transgariepinus* White, p. 69
6. Integument with numerous striae, the arrangement of the striae on the margins differing from those on the rest of the body; venter and posterior margin of dorsum wrinkled; a few indistinct discs on dorsum ... *A. striatus* Bedf., p. 70
   Integument finely wrinkled or corrugated, the margins being similar to the rest of the body; discs larger and more numerous ... ... *A. perengueyi* (Bedf. and Hewitt), p. 72
7. Body broad and rounded in front; discs absent 8
   Body narrower, sub-conical in front, discs present; eyes absent ... ... *A. talaje capensis* (Neu.), p. 74
8. Body with hemispherical granulations[1] 9
   Body with flat contiguous granulations; eyes present. *A. parimentosus* (Neu.), p. 87

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[1] In fully distended adults of *A. moubata* the granulations may be flat.

9. Eyes absent ... ... *A. moubata* (Murray), p. 81
   Eyes present ... ... *A. savignyi* Aud., p. 86

Key to the South African Larvae.

1. Larvae not known ... ... *A. transgariepinus* White.
   A. striatus Bedf.
   Larvae known 2

2. Larvae inactive. Body sub-circular, with 4 pairs of setae on
   dorsum in front : capitulum ventral, hardly visible dorsally.
   A. moubata Murray.
   A. savignyi Aud.
   ? *A. parimentosus* (Neu.)
   Larvae active. Body rarely sub-circular, with the setae on
   dorsum more numerous; capitulum, if ventral, plainly
   visible dorsally 3

3. Setae on dorsum of body present only on the margins; hypo-
   stome dentition 3/3 in front, 2/2 behind.
   A. verspertilionis (Latr.)
   Setae on dorsum arranged otherwise 4

4. Dorsum of body with 10 setae on anterior half and 4 on
   posterior margin, capitulum terminal; palpi elongated with
   4th segment short; hypostome elongated, with dentition
   2/2 ... ... *A. megnini* Dugès.
   Setae on dorsum of body more numerous 5

5. Base of capitulum plainly visible dorsally; hypostome denti-
   tion 2/2 : palpi with 4th segment short.
   A. perengueyi (Bedf. and Hewitt).
   Base of capitulum hardly if at all visible dorsally; hypostome
   dentition otherwise ..., ... 6

6. Palpi and hypostome very long, the dentition of the latter 5/5
   in front, then one row of 3/3 followed by 2/2 behind; palpi
   with 4th segment short ... ... *A. talaje capensis* (Neu.).
   Palpi elongated with 4th segment long; hypostome shorter,
   with dentition as in Fig. 15 ... ... *A. persicus* (Oken.).

1. Argas vespertilionis (Latreille).

" The Bat Tick ".

Carios vespertilionis Latr., 1796, *Précis Caract. Ins.*, p. 177.
Acarus fisheri Savigny, 1826, *Desc. de l'Egypte. Arachnides*, pl. 9,
   f. 6 (1, 1', 2, 2').
Acarus pipistrellae Audouin, 1827, *Desc. de l'Egypte*, etc., éd. 2,
   XXII, Zool., p. 427.
Caris elliptica Kolenati, 1857, *Die Parasit der Chiroptera*, p. 16.
Caris longimani Kolenati, 1857, *ibid.*, p. 16.
Caris decussata Kolenati, 1857, *ibid.*, p. 16.
Caris inermis Kolenati, 1857, *ibid.*, p. 16.
Argas pulchella George, 1876, *Journ. Quekett microc. Club*, IV,
   p. 224.
Argas vespertilionis (Latr.) Neumann, 1896, *Mém. Soc. Zool. de
   France*, IX, p. 20, f. 22-26.

Argas vespertilionis (Latr.) Nutt., Warb., Cooper and Robinson, 1908, *Ticks : Mon. Ixod.*, i, p. 34, t.f. 48-57, pl. 1, f. 4, 5.

Argas vespertilionis (Latr.) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 79, pl. 1, figs. h-p : pl. 2, figs. p-w.

Argas vespertilionis (Latr.) Dönitz, 1910, *Die Zecken Südafr.*, p. 411.

Argas vespertilionis (Latr.) Patton and Cragg, 1913, *A Textb. of Med. Ent.*, pp. 582, 584, pl. 74, f. 5.

Argas vespertilionis (Ltr.) Neumann, 1911, *Das Tierreich : Ixod.*, p. 120, f. 66.

Argas vespertilionis (Latr.) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 282.

Adults (Figs. 8-9).—*Body* broader than long, pointed in front, measuring (excluding hood) $6\cdot8 \times 7\cdot2$ mm. to $7\cdot2 \times 8\cdot7$ mm.; the males being about as large as the females. The dorsum is irregularly convex in the middle, and the lateral margin reflexed. Colour reddish-brown except on the margin which is yellowish-brown. The hood projects forwards in front and is visible dorsally.
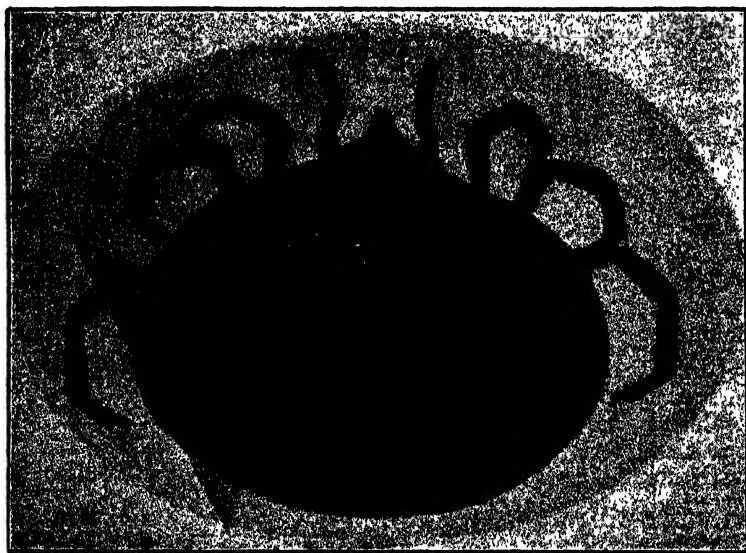
Fig. 8. *Argas vespertilionis* (Latr.), dorsum of ♀.
[*Photo T. Meyer.*

Integument with fine granulations and numerous small discs arranged more or less in lines extending from the lateral margins towards the centre. *Venter*: genital opening between coxae i; spiracles small, situated laterally to coxae iv. A short distance behind the anus there is a pair of organs, each consisting of a narrow, deep, transverse cleft lying in a small area free from granulations; the area above the cleft is finely striate at right angles to the cleft, whilst that behind is finely punctate. *Eyes* absent. *Capitulum* lying in the hood; the base is much longer than broad, and the appendages are extremely small. *Hypostome* narrow, indented at the apex with small marginal teeth. *Palpi* with the first segment much larger than the others, which are very small and narrow. *Legs* long and slender, yellow.
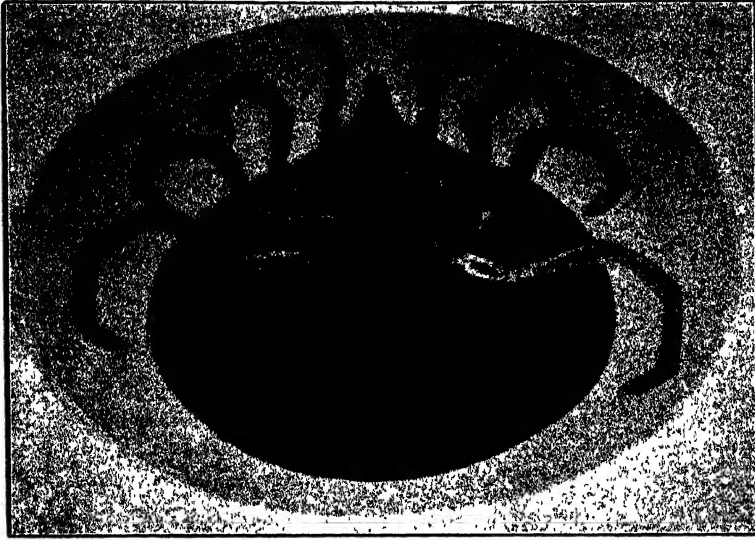
Fig. 9. *Argas vespertilionis* (Latr.), venter of ♀.

[*Photo* T. *Meyer.*

Nymphs (Fig. 10).—There are two nymphal stages, the *second stage* differing from the adults in having either no sexual orifice or only a rudimentary orifice, and the hypostome dentition is 2/2 with 5 or 6 teeth per file. We have specimens measuring 5 × 5·6 mm. and 2·9 × 3·1 mm.

In the *first nymphal stage* the body is almost circular, being usually slightly longer than broad, measuring 2·4 × 2·1 mm. The hood is absent, but the hypostome and palpi are partly visible dorsally. Integument finely creased in zig-zags and margin with irregular folds; both dorsally and ventrally there are numerous small depressions. Organs posterior to the anus present as in the adults.
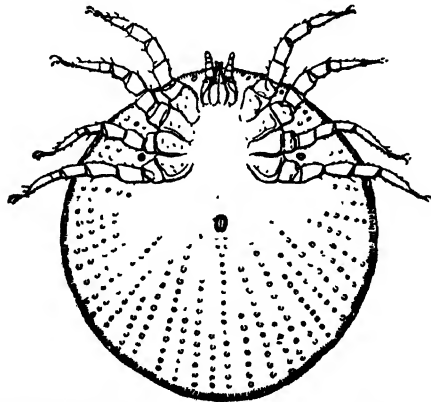
Fig. 10. *Argas vespertilionis* (Latr.), venter of nymph (After Neumann).

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Larva (Fig. 11).—Body of partly and fully fed specimens short oval, $1\cdot3 \times 1$ mm. to $2 \times 1\cdot5$ mm. On the dorsum there are three pairs of setae on the anterior portion, twenty on the margin, and about ten radiating furrows on the posterior half; between the intestinal caeca are radiating series of discs. Integument with fine transverse parallel striae, except anterior to the middle of the dorsum, where there is an oval plate. *Capitulum* almost entirely visible dorsally. *Hypostome* long, narrow and pointed; dentition 4/4 in front, 2/2 behind. *Palpi* slender, with segment 1 short, segments 2 and 3 long, sub-equal, segment 4 short. *Legs* pale and slender, sub-equal; tarsi with a distinct pulvillum.
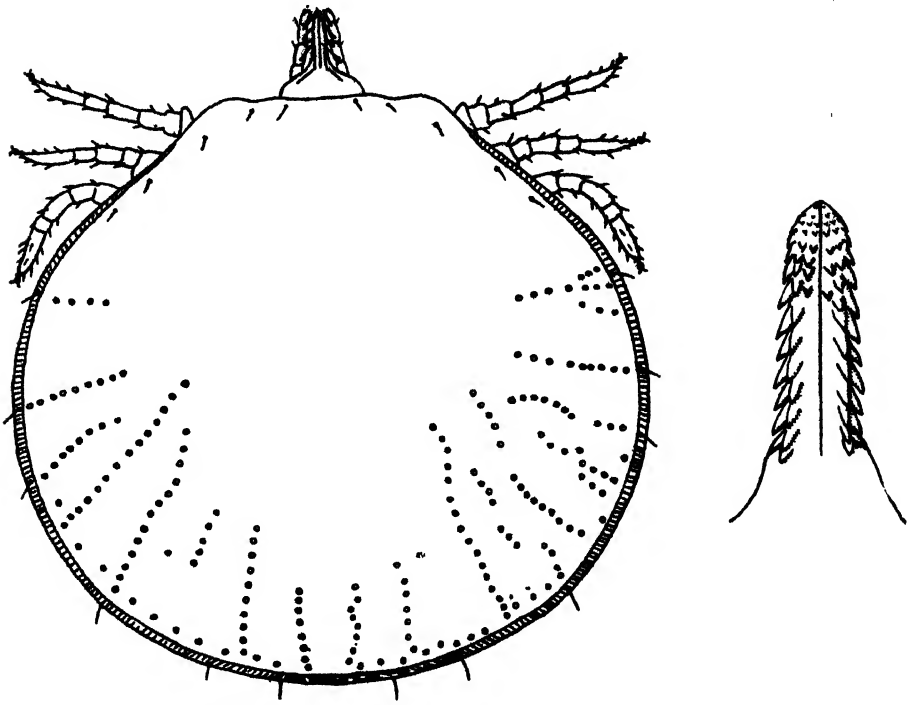
Fig. 11. *Argas vespertilionis* (Latr.), engorged larva and hypostome. G. A. H. B. del.

Hosts and Distribution.—Recorded taken off various species of bats in South Africa, Egypt, Sudan (King), Tunis, England, France and India (Patton and Cragg). Howard (1908) recorded it from *Miniopterus natalensis* (= *M. schreibersi*), Pretoria. Larvae have been taken off *Eptesicus capensis* at Onderstepoort and at Driefontein, S. Rhodesia. It has occasionally been known to attack human beings living in Pretoria in houses frequented by bats, and a specimen has been received taken off a cat at Vryburg, C.P. A nymph was found on a sheep at Onderstepoort. It was kept in a shed which was no doubt visited at night by bats. Latreille's type was taken off *Vesperugo noctula*.

Life-cycle.—The life history has been studied by Patton (Patton and Cragg, 1913). The eggs, which are laid in the resting places of bats, take from 10 to 12 days to hatch. The larvae remain attached to the wing of their host for 10 days and about 24 hours before leaving their host become flat. They moult into first stage nymphs about 5 days after dropping off. The first nymphal stage lasts about 11 days, the ticks taking about 25 minutes to gorge. The second nymphal stage lasts about 17 days, the ticks taking about 50 minutes to feed to repletion. The females can be fertilised immediately after the last ecdysis, and, if fed 22 days later, will commence laying eggs 28 days after moulting.

Transmitter of Disease.—In Tunis the tick has been demonstrated to transmit a spirillum. *Treponema vespertilionis*, to bats.

2. **Argas persicus** (Oken).

" The Fowl Tick ".

Rhynchoprion persicum Oken, 1818, *Isis*, p. 1567, pl. 19, f. 1-4.

Argas mauritianus, Guérin-Méneville, 1829, *Iconogr. du régne animal de G. Cuvier. Arach.*, pl. 6, f. 3.

Argas miniatus C. L. Koch, 1844, *Arch. f. Naturg.*, X, i, p. 219.

Argas americanus Packard, 1872, *U.S. Geolog. Surv. of the territory, etc.*, p. 740, f. 68.

Argas sanchezi A. Dugés, 1891, *La Naturaleza* (2), 1, p. 20.

Argas chinche Goudet, referred to by Neumann, 1901, p. 344, to *A. miniatus.*

Argas radiatus, Railliet, 1893, *Traité de zool. méd., etc., agric.*, fasc. 1, p. 718.

Argas miniatus firmatus Neumann, 1896, *Mém. Soc. Zool. de France*, IX, p. 12.

Argas miniatus (Koch) Salmon and Stiles, 1900, *Ann. Rep. Bur, Anim. Indust. U.S. Dept. Agric.*, XVII, p. 402, t.f. 56-58, 71-81, pl. 78; also Reprint, 1902.

Argas persicus (Oken) Lounsbury, 1903, *Agric. Journ.*, Capetown, XXIII, p. 261, 3 pls.; also Reprint.

Argas persicus (Oken) Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.*, i. pp. 8, 81, t.f. 1-26, pl. 1, f. 3.

Argas persicus (Oken) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 76, pl. 1, figs. f-i, pl. 2. figs. c-n.

Argas persicus (Oken) Dönitz, 1910, *Die Zecken Südafr.*, p. 409.

Argas persicus (Oken) Neumann, 1911, *Das Tierreich. Ixod.*, p. 121.

Argas persicus (Oken) Patton and Cragg, 1913, *A Textbook of Med. Ent.*, pp. 581, 583, pl. 74, f. 1, 2; pl. 75, f. 1; pl. 83, f. 1-3; pl. 84, f. 3; pl. 86, f. 1-3, 5-6.

Argas persicus (Oken) Robinson and Dividson, 1913-1914, *Parasit.*, VI, i, pp. 20-48, t.f. 1-2, pl. 1-6; *ibid.*, VI, iii, pp. 217-256, t.f. 1-8, pl. 14-17; *ibid.*, VI, iv, pp. 383-424, t.f. 1-8, pl. 25-28.

Argas persicus (Oken) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 281, f. 6.

ADULTS (Figs. 12-14).—Females measure from 7 × 5 mm. to 12·5 × 8·5 mm., and males from 4·25 × 2·5 to 8 × 5 mm. *Body* yellowish-brown to slate coloured; oval in shape, being widest towards the posterior end. *Integument* with numerous oval or round discs, arranged more or less symmetrically. *Venter*: genital opening between coxae i and ii; spiracles small, crescentic; eyes absent. *Capitulum* with four long setae directed forwards on the base, two post-hypostomal, one near the articulation of each palp. Palpi about twice a slong as the hypostome. *Hypostome* with several fine denticles on each side distally, followed by stout teeth 2/2, the teeth increasing to 3/3, 4/4, 5/5 basally, but decreasing in size. *Legs* sub-equal and similar, pale yellow. Coxa i separated from coxa ii in female; coxae ii, iii and iv contiguous.
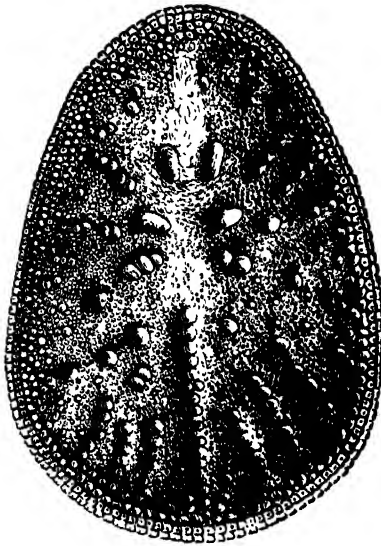
Fig. 12.   Fig. 13.

Fig. 12. *Argas persicus* (Oken), dorsum of ♀.
C. G. Walker del.

Fig. 13. *Argas persicus* (Oken), venter of ♀.
C. G. Walker del.

NYMPHS.—There are two nymphal stages. The second-stage nymph only differs from the adults in having no sexual orifice, and the discs are not quite as numerous. When full-fed it measures 5·5 to 7 mm. The first-stage nymph differs from the second-stage nymph when fed in having slightly fewer discs, and in unfed specimens the discs are absent.

LARVA (Fig. 15).—The unfed larva measures 0·67 × 0·65 mm. to 0·73 × 0·71 mm. It is pale in colour, almost spherical in shape, with the capitulum inserted ventrally, but projecting well in front of the body. Integument finely striated, except in the middle of the dorsum where there is a semi-circular plate. Setae more numerous on the body than in other known larvae. *Legs* long, the tarsi with a distinct pulvillum. After feeding they become dark and visibly swollen.

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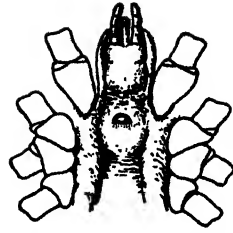
Fig. 14. *Argas persicus* (Oken).

C. G. Walker del.

ANATOMY.—Both the external and internal anatomy of *A. persicus* have been studied by Robinson, L. E., and Davidson, J. (1913, 1914), and also by Patton and Cragg (1913).

REGENERATION.—Regeneration in *A. persicus* has been studied by Hindle and Cunliffe (*Parasit.*, 1914, VI, iv, pp. 353-371, t.f. 1-4) and Nuttall (*Parasit.*, 1920, XII, i, pp. 7-26, t.f. 1-4).

HOSTS.—It is mainly parasitic on fowls but also attacks ducks, geese, turkeys, pigeons, canaries and ostriches. Howard (1908) also recorded it from secretary bird (*Sagittarius serpentarius*), and specimens have also been taken off wild guinea-fowl (*Numida papillosa transvaalensis*) at Pienaars River, Tvl. It has frequently been reported to attack man in Persia, but rarely does so in South Africa.
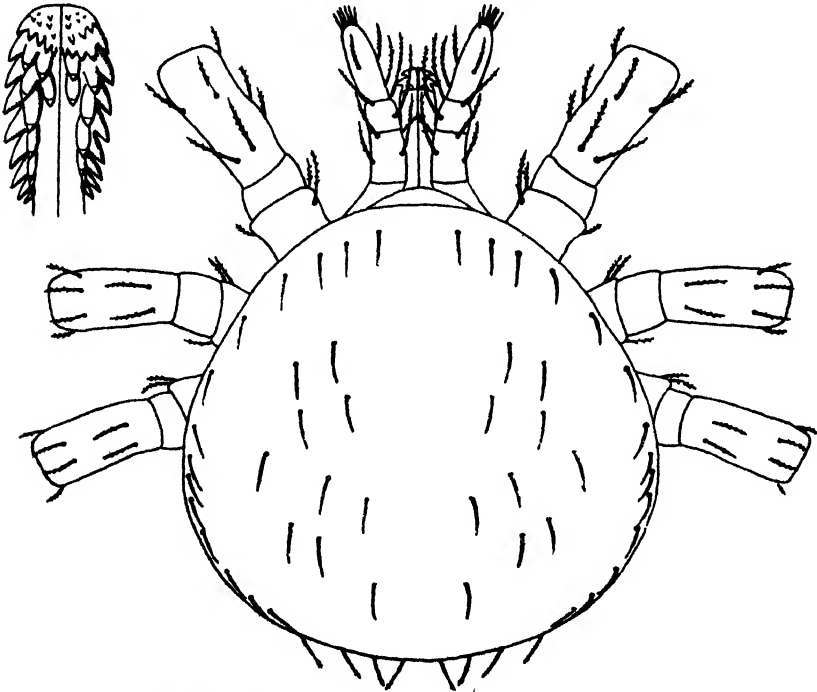
Fig. 15. *Argas persicus* (Oken), unfed larva and hypostome

G. A. H. B. del.

GEOGRAPHICAL DISTRIBUTION.—It is very common throughout South Africa, and is the most serious pest the poultry-keeper has to contend with. It has also been recorded from Southern Rhodesia, Mozambique, Belgian Congo, Egypt, Sudan, Algeria, Mauritius, Russia, Turkestan, Persia (type locality), India, China, the southern part of North America, South America and Australia.

LIFE CYCLE.—The life cycle has been studied by Lounsbury (1903) in the Cape, Nuttall (1908) at Cambridge, and other workers.

The *eggs* are usually laid in batches of about 20 to 100 in the cracks and crevices of walls of fowl-houses, etc., or under the bark of trees. Fuller (1896) and Brown (1902), however, have observed oviposition taking place occasionally on fowls in Australia. The eggs usually take about three weeks to hatch, the time varying according to the temperature. The *larvae* crawl about in search of a host as soon as their chitin has hardened, and on getting on to a suitable host, they immediately attach themselves to its skin and commence to suck blood, continuing to feed for 5 to 10 days, according to the temperature. They then drop off and seek shelter, moulting into nymphs about 8 days later in summer.

The *nymphs* and *adults*, unlike the larvae, only feed on their hosts at intervals, hiding in the crevices of woodwork, etc., after feeding. They invariably suck blood at night, but have been known to feed during the day when protected from strong light. They are rapid feeders, usually only taking from 30 minutes or less to two hours to gorge themselves. The first nymphal stage lasts about two to three weeks, and then they moult into the second nymphal stage, and again, after about five weeks, into adults. The females feed more plentifully than the males; usually about once a month during the summer. About a week after each meal they lay a batch of eggs. Lounsbury found that the complete life-cycle from egg to egg stage occupied about ten months in the Cape. The larvae are only able to live for seven or eight weeks without food, but nymphs have been known to live for a year without having had a meal, and the adults for two to three years.

The sensory perceptions of *persicus* have been studied by Hindle and Merriman (*Parasit.*, 1913, V, pp. 203-216, f. 1-2).

TRANSMITTERS OF DISEASE.—The fowl tick is the chief transmitting agent of the fowl spirochaete, *Treponema anserinum* Sakharoff (= *T. gallinarum* Marchoux and Salimbeni), which is usually fatal to birds, as was first demonstrated by Marchoux and Salimbeni in Brazil in 1903. It also produces fatal results in geese, ducks, guinea-fowls, turkeys, canaries, turtle-doves, and other birds. The disease usually breaks out four to five days after the ticks bite the birds. The ticks become infected by sucking the blood of infected birds, and when infection has taken place and the ticks remain at a favourable temperature (30-35° C), the organisms multiply within the bodies of the ticks. Should, however, the ticks be kept at a low temperature (15 to 18° C.) the organisms seem to disappear, but reappear when the ticks are again maintained at a suitable temperature. Once the ticks have become infected they are able, whenever the temperature is suitable,

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to transmit the disease to healthy fowls for six months or even longer. The infection may pass through the egg to the next generation, and, as Hindle (1912) has demonstrated, this generation may, without re-infection, hand it on to the next.

Since coccoid bodies may be found within the lumen of the gut, sexual organs, malpighian tubes and in the excreta of infected ticks, but soon disappear from the salivary glands, it would appear that the disease is transmitted in the same way that *T. duttoni* is transmitted by *A. moubata*, namely: In the act of feeding, the tick may void excrement and exude a few drops of secretion from the coxal glands situated in the first intercoxal space, which dilutes the excrement and facilitates its getting into the wound caused by the mouth-parts of the tick.

The disease is fairly common in the Union, and prevalent in Rhodesia. It also occurs in other parts of Africa, S.E. Europe, Asia, S. America and Australia. The fowl tick also does a considerable amount of harm to birds, especially when very numerous, by sucking blood and the irritating effects it produces, which result in lowering the vitality of infected birds. Birds kept in badly infected houses and runs cease to feed normally, rapidly fall off in condition, and may eventually die of anaemia and general debility.

Aegyptianella pullorum Carpano in Fowls.—Bedford and Coles (1933) demonstrated that *A. persicus* is a transmitting agent of this disease. The disease was transmitted to nine healthy chickens by single adult ticks which had previously fed on infected birds. Moreover, it was shown that the ticks retain their infection after feeding on healthy birds, as one adult tick transmitted the disease to two healthy chickens. The shortest interval between feeding on an infected bird and healthy chicken was 26 days. The incubation period in chickens after an infected tick has bitten varies from 12 to 15 days or more.

The tick has also been reported to cause prolonged illness and even death to man in Persia. It frequently became so numerous in houses in that country as to necessitate the removal of entire villages to new sites. It has also been recorded from houses in Egypt.

3. **Argas transgariepinus** White.

Argas transgariepinus White, in Methuen, 1846, Life in South Afr., p. 318, pl. 2, f. 4.

Argas kochi Neumann, 1901, *Mém. Soc. zool. France*, XIV, p. 254.

Argas transgariepinus (White) Neumann, 1906, *Arch. de Parasit.*, X (2) ,p. 218.

Argas transgariepinus (White) Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.*, i, p. 29, f. 36-37.

Argas transgariepinus (White) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 81.

Argas transgariepinus (White) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 282.
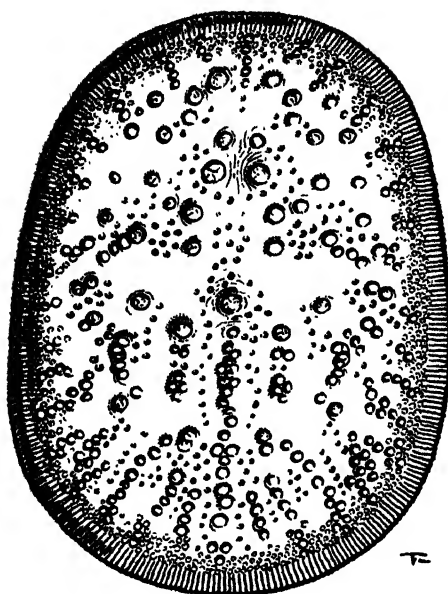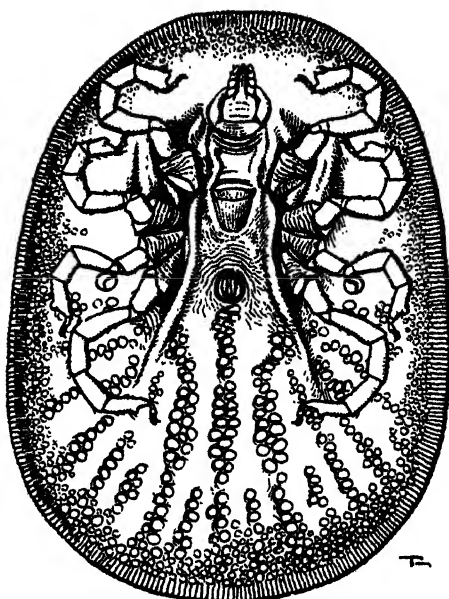
Fig. 16.   Fig. 17.

Fig. 16. *Argas transgariepinus* White, dorsum of ♀.
Fig. 17. *Argas transgariepinus* White, venter of ♀.
(Drawn from type in British Museum.)

A. J. E. Terzi del.

Following description after Nuttall and Warburton :—

ADPLTS. (Figs. 16, 17).—Body short, oval and almost as broad in front as behind. Margin striate like that of *reflexus*, but relatively broad (3 mm.). Spiracles scarcely as long as the width of the anus. Sexual orifice of the male ver small, facing the posterior extremity of coxa i. Capitulum very small (0·8 mm.) and very posterior, far from coxae i, lying in a clearly marked camerostome. Hypostome narrow, palps short. Coxa i far from coxa ii, and the space between the coxae on the two sides very broad—equal to one-third the width of the venter. Tarsi i much humped distally, the protuberances on the other tarsi slight. Females measure from 8·5 × 6 mm. to 10 × 7 mm.

The types of *A. transgariepinus*—three females in the British Museum, differ from *A. kochi*, based on a single ♂ from Basutoland in the Paris Museum, in having the tarsal protuberance almost as well marked on tarsi ii, iii, and iv as on tarsi i.

4. **Argas striatus** Bedford.

Argas striatus Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 221, f. 1-2; *ibid.*, p. 282.

FEMALE.—*Body* long and narrow, 6 × 3·5 mm., the anterior margin tapering to a rounded point, the sides parallel, and the posterior margin rounded. Margin with a few short, pale setae, transversally striated and raised higher than the rest of the integument in unfed specimens; brown in colour and paler than the rest of the body which is dark brown. Dorsum striated, the enclosed areas between the striae small and irregular in shape; on the anterior and median

portions of the body they are flat and smooth, and behind raised, making the surface appear wrinkled; in the middle a semi-circular row of four shallow discs and a median one beneath them; grooves present as shown in figure. *Venter* wrinkled, without discs; genital orifice situated between coxae i; median post-anal groove long and very shallow; a deep groove extends on each side from coxae iv almost to the posterior margin, the inner margin being raised higher than the rest of the integument. *Hypostome* with minute teeth anteriorly, followed by dentition 2/2. *Legs* yellowish; coxa i separated from coxa ii; coxae ii, iii and iv contiguous; tarsi without protuberances.
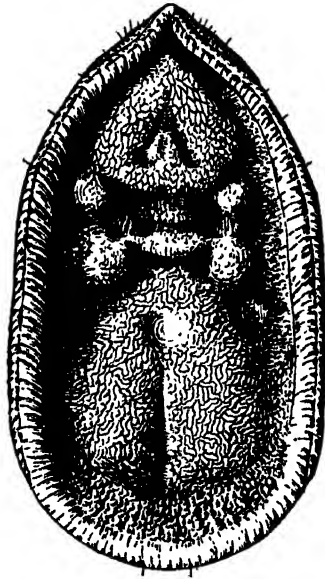
Fig. 18. *Argas striatus* Bedford, dorsum of ♀.
C. G. Walker del.

The description is based upon three unfed females found in the nest of a sociable weaver, *Philetairus socius* (Lath)., at Kenhardt, C.P. They were sent by Dr. R. F. Lawrence. The holotype and one paratype in the South African Museum, Capetown, and the other paratype in the Onderstepoort collection.

This species is apparently closely related to *A. aequalis* (Neumann), which was described from a single late-state nymph collected in Tanganyika Territory. The host is unknown. As Neumann did not figure the species, and his description is somewhat short, it is difficult to compare it with the specimens described above, but in *aequalis* the integument is folded, with very fine granulations.

As sociable weavers occupy the same nests year after year it is possible that *A. striatus* may be a temporary feeder, remaining in the nests and hibernating during the periods the nests are not occupied by the birds. The South African cliff swallow (*Petrochelidon spilodera*) is another bird which uses the same nest year after year, and *A. perengueyi* (Bedford and Hewitt), which is parasitic on this species, hibernates in the nest during the winter months.

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5. Argas perengueyi (Berford and Hewitt).
" The Swallow Tick ".

Ornithodorus perengueyi Bedf. and Hewitt, 1925, *S. Afr. Journal. Nat. Hist.*, V, i, p. 259, pl. 19, f. 1-3.

Argas perengueyi (Bedf. and Hewitt) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 281.
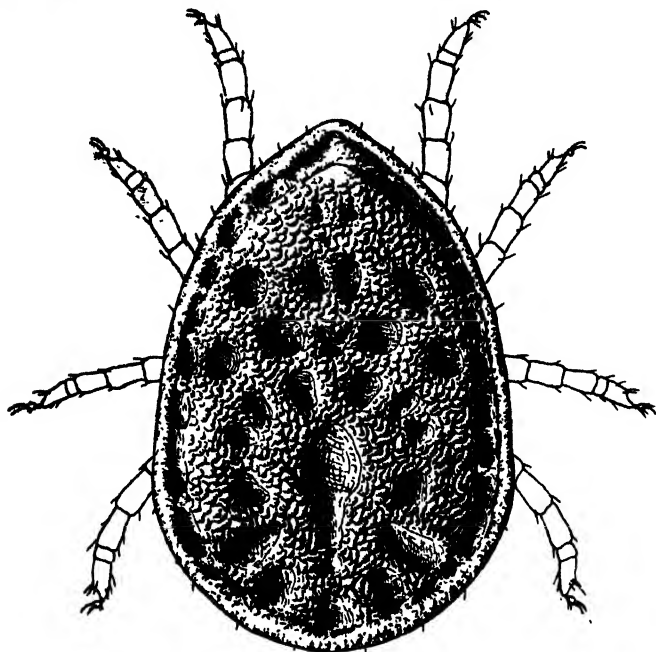
Fig. 19. *Argas perenqueyi* (Bedford and Hewitt), dorsum of ♀.
C. G. Walker del.

ADULTS. (Figs. 19-21).—Body of female 4·1 to 6·5 mm. long, 2·6 to 4 mm. broad; of male 3·5 to 4·5 mm. long, 2·1 to 3 mm. broad; conical anteriorly, rounded posteriorly with sides almost straight and parallel; brown or slate-coloured, with pale yellowish lateral margins. *Integument* very finely and closely wrinkled or corrugated. *Dorsum* with symmetrically arranged depressed areas and lateral grooves; sparsely clothed with minute pale setae. The lateral grooves are frequently more or less broken and discontinuous, and sometimes indistinct. *Venter* clothed with minute pale setae as on the dorsum, and with three depressions on each side of the median postanal groove; transverse pre-anal and post-anal grooves absent; supra-coxal folds well developed. Genital orifice situated between coxae i. *Spiracles* small, circular. *Eyes* absent. *Capitulum* lying in a deep camerostome; base slightly broader than long, with a pair of long admedian setae directed backwards. *Hypostome* (fig. 21) with asymmetrically arranged teeth. *Palpi* broad, the second segment being the largest, the third shorter and broader than the fourth. *Legs* pale yellow; coxae i larger than the others, separated from coxae ii; coxae ii, iii, and iv contiguous; tarsi i with two protuberances; tarsi ii to iv with only one protuberance.

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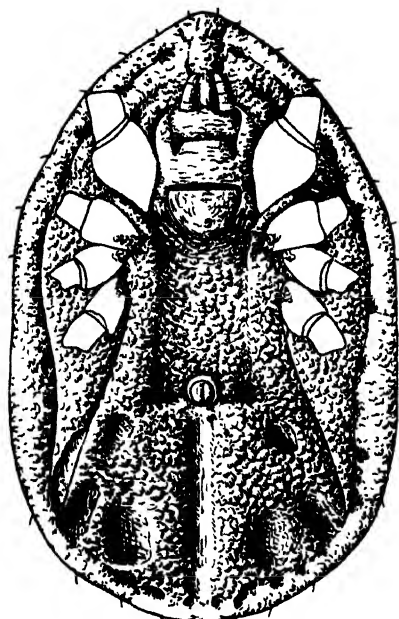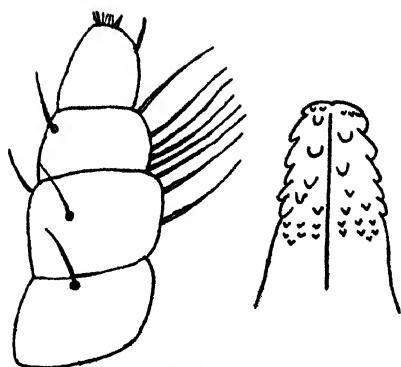
Fig. 20.

Fig. 21.

Fig. 20. *Argas perengueyi* (Bedford and Hewitt), venter of ♀.

C. G. Walker del.

Fig. 21. *Argas perengueyi* (Bedford and Hewitt), left palpus and hypostome

G. A. H. B. del.
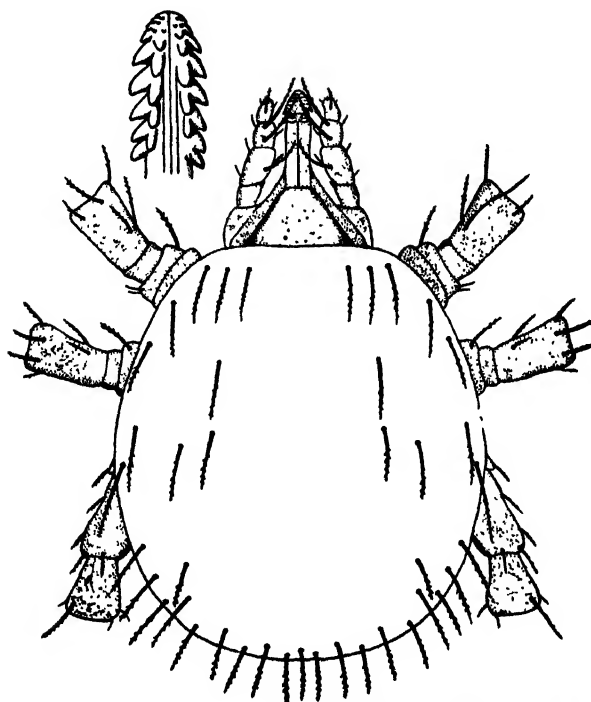
Fig. 22. *Argas perengueyi* (Bedford and Hewitt), unfed larva and hypostome
G. A. H. B. del.

NYMPHS.—It is not known how many nymphal stages there are. They resemble the adults except for the absence of the sexual orifice. Body from 3×2 mm. to $3 \cdot 9 \times 2 \cdot 5$ mm.

LARVA (Fig. 22).—The unfed larva measures $0 \cdot 64 \times 0 \cdot 54$ mm. It is pale in colour, and has the capitulum terminal. *Body* with numerous setae. *Palpi* of medium length, the second segment the largest, the fourth segment small. *Hypostome* of medium length; dentition 2/2. *Tarsi* with a distinct pulvillum.

HOSTS AND DISTRIBUTION.—Described from adults and one nymph taken at Nqamakwe, C.P. They were collected by the Rev. L. S. Byrde, who reported on them as infesting a native church. I have since taken adults and immature forms in the nests of *Petrochelidon spilodera* (South African cliff swallow) at Onderstepoort. The birds return to the same nesting sites year after year, and the ticks may be found in the nests throughout the winter months. Specimens have also been fed on fowls.

Transmitters of Disease.—Bedford and Coles (1933) failed to transmit *Aegyptianella pullorum* Carpano to fowls by feeding two or three adults of *A. perengueyi* on healthy chickens after they had fed on infected birds.

6. **Argas talaje capensis** (Neumann).

" The Penguin Tick ".

Ornithodoros talaje var. *capensis* Neumann, 1901, *Mem. Soc. zool. France*, XIV, p. 258.

Ornithodoros talaje var. *capensis* (Neu.) Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.* i, p. 61, t.f. 58, 89, pl. 3; f. 1-2.

Ornithodoros talaje var. *capensis* (Neu.) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 88, pl. 1, figs. r, s; pl. 3, figs. a-e.

Ornithodorus talaje Dönitz, *Die Zecken Südafr.*, 1910, p. 416, pl. 16A, f. 6, 7 *nec* Guérin-Méneville, 1849.

Argas talaje capensis (Neu.) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 280.

This variety closely resembles the type which occurs in native houses in South America.

ADULTS (Figs. 24; 25).—*Body* of female 5 to 6 mm. long, 3 to 3·5 mm. broad; of male 3·5 to 5 mm. long, 2 to 3 mm. broad; conical anteriorly, rounded posteriorly with sides usually almost parallel; dirty yellow changing to yellowish or reddish-brown when gorged. *Integument* with numerous large, sub-equal mammillae, and a number of large discs on the dorsum. When unfed there is a lateral groove on each side on the inner margin of the dorsal surface, which is thick and raised, and other depressions are also present on the dorsum, but these disappear in replete specimens. *Venter*: genital
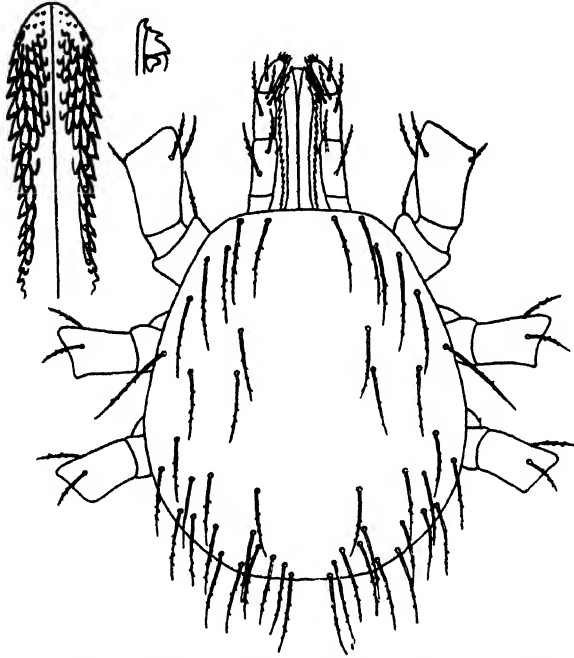
Fig. 23. *Argas talaje capensis* (Neu.), unfed larva, hypostome and digit. G. A. H. B. del.

opening opposite posterior margin of coxae i; coxal folds well-developed, extending between coxae i and ii; pre-anal and post-anal grooves present, the latter with transverse striae. *Spiracles* conical, situated on the dorsal surface of the supra-coxal folds. *Eyes* absent. *Capitulum* lying in a deep camerostome, with prominent lateral flexible flap-like borders which protect the capitulum; base finely wrinkled transversely. *Hypostome* indented at apex, dentition 2/2, except at the tip where there are a number of small teeth. *Palpi* with segment i the longest, slightly longer than segment ii; segment iii shorter than segment iv; numerous setae present on all segments. *Legs* pale brown, long and slender with numerous fine setae; coxae contiguous decreasing in size from pair i to iv, covered with small granulations; tarsi tapering without marked protuberances.

NYMPHS.—It is not known how many nymphal stages there are. They are yellowish-brown in colour and resemble the adults except for the absence of the sexual orifice. Body 1·73 × 1·0 mm. to 2·42 × 1·43 mm.
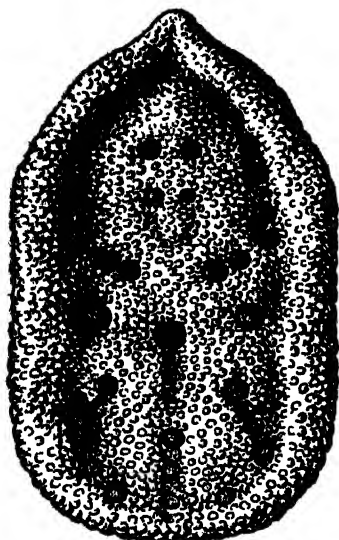
Fig. 24.
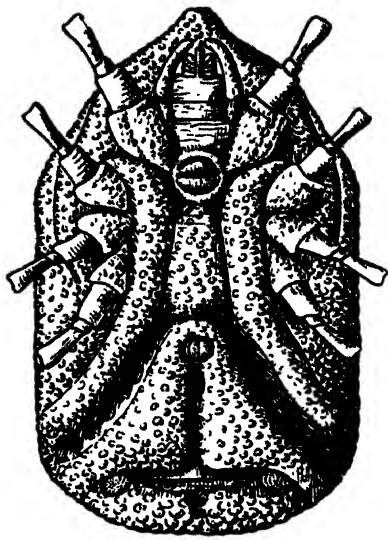
Fig. 25.

Fig. 24. *Argas talaje capensis* (Neu.), dorsum of ♀.
C. G. Walker del.

Fig. 25. *Argas talaje capensis* (Neu.), venter of ♀.
C. G. Walker del.
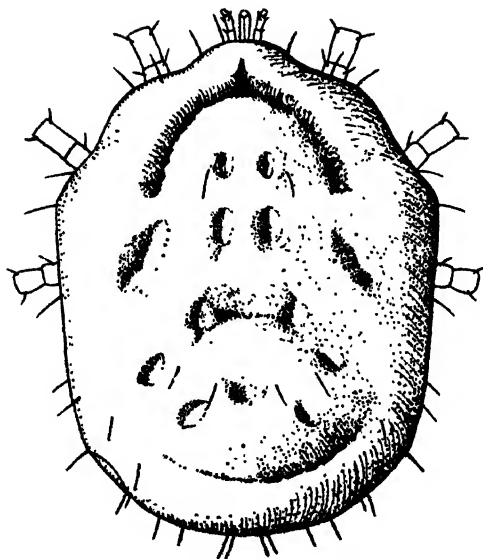
Fig. 26. *Argas talaje capensis* (Neu.), dorsum of engorged larva.
C. G. Walker del.

LARVA (Figs. 23, 26).—The unfed larva measures 0·47 × 0·38 mm. It is pale in colour, and has the capitulum inserted ventrally, the base being hardly if at all visible from above. *Body* with numerous setae, resembling that of *A. perengueyi* in shape; plate on dorsum absent. *Palpi* elongated, first segment long, only the apical portion is shown in the figure; fourth segment short. *Hypostome* very long, dentition 5/5 in front, then one row of 3/3 followed by 2/2. *Tarsi* with a distinct pulvillum.

HOSTS AND DISTRIBUTION.—This species is common in the nests of the jackass penguin (*Spheniscus demersus*) on the islands off the western coast of the Cape Province, and specimens have been taken in the nests of the same birds on St. Criox Island off Port Elizabeth (coll. J. Hewitt). It readily attacks both man and fowls when opportunities offer. Nuttall, Warburton, Cooper and Robinson (1908) have recorded specimens collected by the Challenger Expedition in 1876 at St. Paul's Rocks from birds' nests. Neumann (1907) recorded specimens found on the ground at Cargados Carajos (Siren Island), and Howard (1908) states that it has been reported from Tristan de Cunha.

LIFE-CYCLE.—The life-cycle is not known.

7. **Argas megnini** Dugès.

" The Spinose Ear Tick ".

Argas megnini Dugès, 1883, *Naturaleza*, V, p. 195.

" *Argas americana* Packard," Townsend, 1893, *Journ. New York Ent. Soc.*, I, (2), p. 50.

Rhynchoprium spinosum Marx, 1895, *Proc. Ent. Soc. Wash.*, June 24, p. 199, pl. 2, f. 1, li.

Ornithodoros megnini (Dugès) Neumann, 1896, *Mém. Soc. zool. France*, IX (1), p. 42, f. 36a-b.

Ornithodoros megnini (Dugès) Salmon and Stiles, 1900, *Ann. Rep. Bur. Anim. Indust.*, *U.S. Dept. Agric.*, XVII, p. 408, t.f. 94-108, pl. 79; also Reprint, 1902.

Ornithodoros megnini (Dugès) Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.*, i, pp. 71, 103, f. 102-112.

Ornithodoros megnini (Dugès) Bedford, 1912, *Rep. Dir. Vet. Res.*, *Un. S. Afr.*, II, p. 343, pl. 37.

Ornithodoros megnini (Dugès) Bedford, 1925, *Journ. Dept. Agric.*, *Un. S. Afr.*, X, pp. 147-153, f. 1-2, also Reprint No. 7, 1925.

Argas megnini (Dugès) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust.*, *Un. S. Afr.*, XVIII, p. 280.

ADULTS. (Figs. 27, 28).—Females 5 to 10 mm. long, 3 to 5 mm. broad; males slightly smaller. *Body* brown to slate-coloured, yellowish between the post-anal grooves, fiddle-shaped, wider in front than behind, and constricted near legs iv. *Integument* very finely granulated with small circular shallow pits, a short, pale seta arising from each; symmetrical depressions on both dorsum and venter as shown in the figures; between the post-anal grooves there are numerous short spines. Reticulate fossettes occupy the depressions, others scattered here and there. *Spiracles* circular. *Eyes* absent. *Capitulum* very small. *Hypostome* unarmed, broad at the base, rounded distally. *Legs* pale yellow.
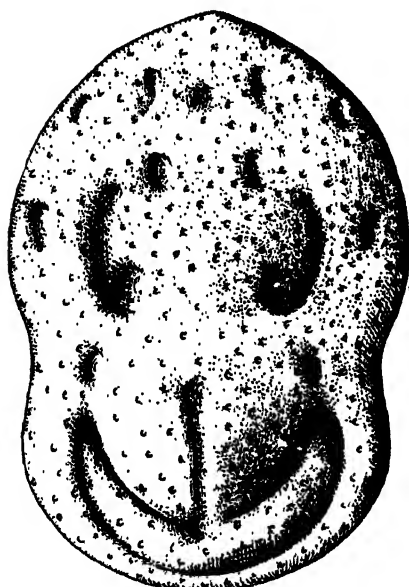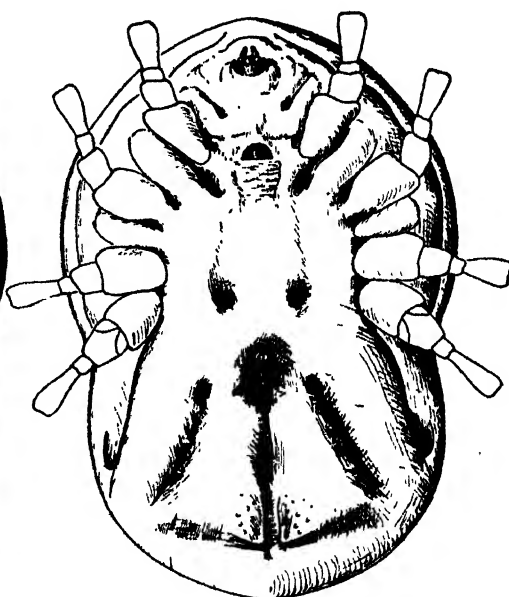
Fig. 27.   Fig. 28.

Fig. 27. *Argas megnini* Dugès, dorsum of ♀.
C. G. Walker del.

Fig. 28. *Argas megnini* Dugès, venter of ♀.
C. G. Walker del.

NYMPH (Fig. 29).—May be distinguished by the integument being beset with numerous backward-projecting spines in front and short setae behind; pits absent; spiracles situated on salient tubercles which project laterally between legs iii and iv.

Body 3-9 mm. long, 2-6 mm. broad; pale when unengorged, reddish when slightly gorged, turning to slate when partly, and yellowish-brown when fully gorged; lozenge-shaped, abruptly constricted at legs iv. *Capitulum* subterminal in unfed nymphs, but usually projecting well beyond margin of body in later stage nymphs. *Hypostome* lanceolate, dentition 4/4, with 7 to 9 teeth per file. *Legs* slightly longer and stronger than in the adults.

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LARVA (Figs. 30, 31).—The unfed larva measures 0.53×0.48 mm. It is pale in colour, oval in shape with the capitulum terminal. The integument is finely striated except in the centre of the dorsum. On the anterior half of the dorsum there are 10 setae, and four shorter ones on the posterior margin. On the venter there are four pairs of setae between the legs and one pair behind the anus. *Hypostome* elongated, with dentition 2/2. When gorged the larva is inactive, this being the " pupa-like stage ". It is usually pale in colour, but may be reddish; oval in shape, with the anterior margin pointed. The legs are very small for the size of the tick, and are difficult to see without the aid of a lens.
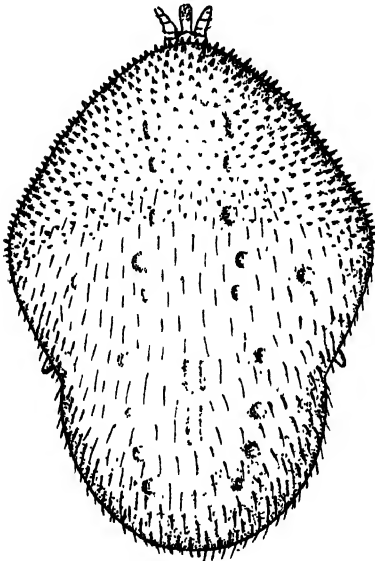
Fig. 29.   Fig. 30.

Fig. 29. *Argas megnini* Dugès, dorsum of partly fed nymph.
C. G. Walker del.
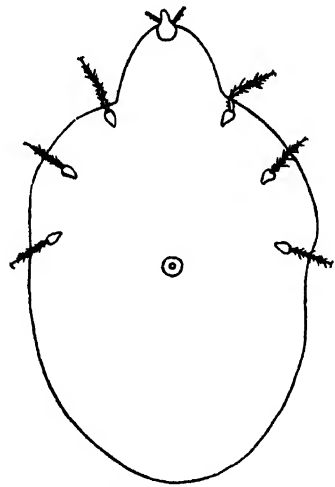
Fig. 30. *Argas megnini* Dugès, venter of gorged larva.
G. A. H. B. del.

HOSTS.—The larvae and nymphs are usually only found in the ears of their hosts. Storey (1920), however, observed on one occasion two almost fully gorged nymphs attached to the bodies of sheep. They are chiefly parasitic on cattle, sheep and goats, but also attack man, horses, donkeys, mules, dogs, cats and ostriches. The adults are non-parasitic.

GEOGRAPHICAL DISTRIBUTION.—This is an American tick which has established itself throughout the arid districts of the Cape Province and Orange Free State. It also occurs in parts of Natal, and in the Transvaal as far north as the Pretoria District. It appears to have been observed by Manley in the Cape Colony as far back as 1898, and was found by Theiler at Vryburg in 1912.

LIFE-CYCLE.—The *larvae* on hatching crawl on to a host and attach themselves to the tender skin inside the ears, usually below the hair-line, and sometimes as far as the eardrum. Here they usually engorge in from 5 to 10 days, when they become quiescent and are unable to move until they have cast their skins and become nymphs. The *nymphs*, in turn, also attach themselves to the skin lining of the ears. The length of time they remain feeding in the ears of their hosts varies considerably. The shortest period in which they can gorge appears to be about a week, but they have been observed at Onderstepoort to remain attached for three months, and according to
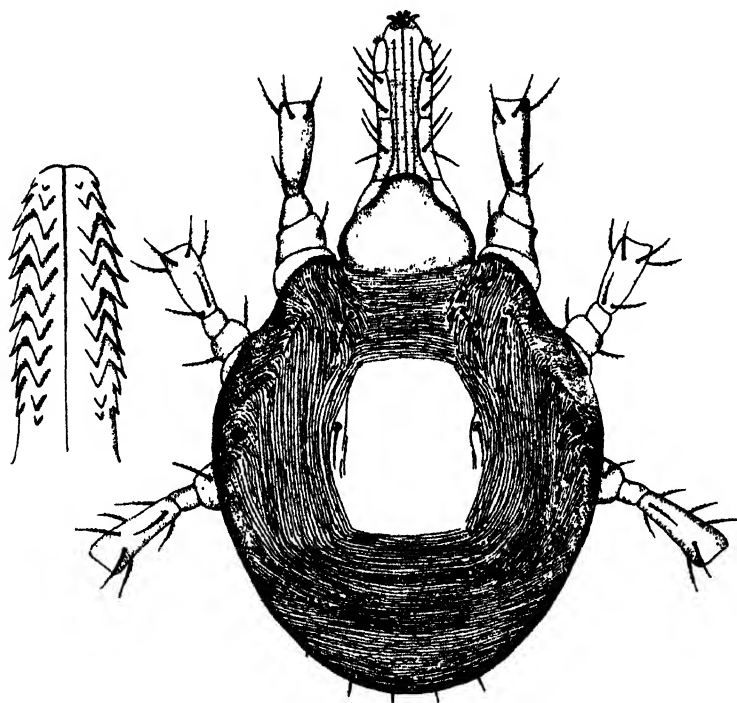
Fig. 31. *Argas megnini* Dugès, dorsum of unfed larva and hypostmoe.
G. A. H. B. del.

American observers may remain in the ears for seven months. However, Storey (1920), who made observations in the Aliwal North District, C.P., states that they generally leave heir hosts within thirty-six days. When full grown the nymphs drop to the ground and crawl about in search of a sheltered spot where they can moult into *adults* and the females lay their eggs. The sites usually chosen by the ticks are the cracks and crevices in the walls of kraals and stables, usually close to the ground. As a rule, therefore, the ticks are only a serious pest on farms where animals are kraaled or stabled. Other sites selected by the ticks are cracks and crevices of posts, vertical rather than horizontal, and adults have also been found under the bark of trees at Onderstepoort.

Under favourable circumstances a female may live up to eleven months awaiting a male, and according to one American observer even as long as eighteen months. After mating has taken place, however, egg-laying commences in about a week and may continue steadily for as long as six months, the eggs being deposited at intervals in small batches. When egg-laying is completed the females die. The *eggs* hatch in twenty-two to fifty-six days. As a rule the *larvae* do not survive longer than a month without food, but under exceptionally favourable circumstances they may be kept alive for four months.

The *adults* are unable to penetrate the skin of animals owing to the fact that their mouth-parts are only partially developed. It is not necessary for them to nourish themselves since enough food is stored up in the nymphal stage to last for adult life and egg-laying. They are, therefore, never found on animals.

TRANSMITTER OF DISEASE.—This tick is not known to transmit disease, but the ticks do a considerable amount of harm to their hosts when numerous by puncturing the tender skin of the ears and sucking blood, at the same time injecting an irritating fluid. The wounds thus caused often ulcerate, and the constant irritation and possible toxic effects of the ticks cause nervous and digestive disturbances, which result in lowering the vitality of the infested animals. The animals cease to feed normally and rapidly fall off in condition. Calves, sheep, and goats not infrequently succumb if sufficiently badly infected. It is not uncommon to find the ear-canals completely filled with a mingled mass of ticks, particles of earwax, and other matter.

Mrs. V., Mooi River, Natal, writes: " I have found three of the ticks in my ears. They caused intense earache, and general disturbances in the head and throat . . . the lining membrane of my ears seems to have been eaten away, causing supperation and blood. They must have been present some time without my realising that they were there and thinking that I had a severe attack of earache ". One specimen was sent which proved to be a partly engorged nymph.

8. **Argas moubata** Murray.

" THE TAMPAN TICK."

Argas moubata, Murray, 1877, *Econ. Ent., Apt.*, p. 182.
Ornithodoros savignyi var. *caecus* Neumann, 1901, *Mém. Soc. Zool. France*, XIV, p. 256.
Ornithodoros moubata (Murray) Dönitz, 1906, *Sitz.-Ber. Ges. naturf. Freunde*, p. 144.
Ornithodoros moubata (Murray), Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.*, i, pp. 46, 96, f. 58, 66-80.
Ornithodoros savignyi var. *caecus* (Neu.) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 86, pl. 1, figs. a-e; pl. 3, figs. g-h.
Ornithodorus moubata (Murray) Dönitz, 1910, *Die Zecken Südafri.*, p. 415.
Ornithodoros moubata (Murray) Neu., 1911, *Das Tierreich, Ixod.*, p. 123.
Ornithodorus moubata (Murray) Patton and Cragg, 1913, *A Textbook of Med. Ent.*, p. 588, pl. 74, f. 6.
Argas moubata (Murray) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 280.

ADULTS. (Fig. 32).—Body oval, broadly rounded in front and behind and slightly constricted on a level with coxae iii and iv, usually measuring from 4 × 2·6 mm. to 12 × 10 mm., but we have one gorged female 15·5 × 12 mm. Colour usually brown with occasional dull ochreous patches, darker in gorged and preserved specimens. *Integument* mammillated, except along shallow grooves which disappear in gorged specimens, but remain recognisable by the absence of mammillae; short, pale setae present between the mammillae, more numerous in front. *Venter* with well-developed coxal folds and preanal groove, and three pairs of long furrows behind, also a short median depression. *Spiracles* crescentic, situated above the supracoxal folds. *Eyes* absent. *Capitulum* free. *Palpi* with the first two segments equal in length, the third the shortest. *Hypostome* dentition 3/3 in front, the teeth smaller and more numerous posteriorly. *Legs* yellowish, well-developed, the fourth pair the longest. Coxae contiguous and diminishing from i to iv. Tarsi and protarsi humped.
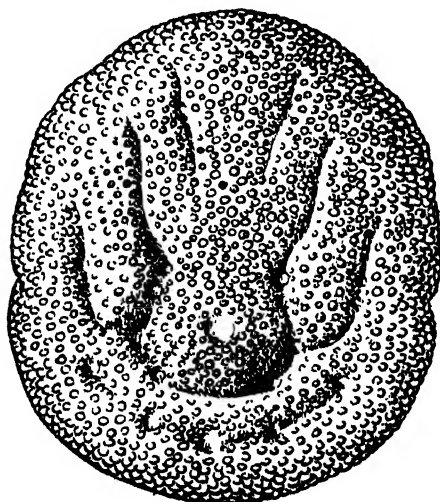
Fig. 32. *Argas moubata* Murray, dorsum of ♀.
O. G. Walker del.

NYMPHS.—The male nymphs undergo 3 to 5 and the female nymphs 3 to 7 ecdyses before moulting into adults. They resemble the adults, except for the absence of the sexual orifice, and the hypostome dentition, the teeth increasing, as Cunliffe has shown, each time the tick undergoes ecdysis. The first stage nymph measures 1·38 × 1.12 mm. when unfed, and from 1·5 × 1·2 mm. to 1·78 × 1·41 mm. after feeding. Full grown nymphs may attain the size of small adults.

LARVA. (Figs. 33, 34).—Body brown, sub-circular, measuring from 1·26 × 1·06 mm. to 1·34 × 1·13 mm. When developed the mammillae on the nymphal integument can be plainly seen. Hypostome dentition 1/1 (two files).

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HOSTS.—It is chiefly parasitic on man, but also attacks domestic animals, rabbits, rats, mice and fowls. Adults and nymphs have also been taken off 44 tortoises (*Testudo oculifera* and *T. verreauii*), Niekerk's Hope, Kimberley, C.P. (coll. J. H. Power).
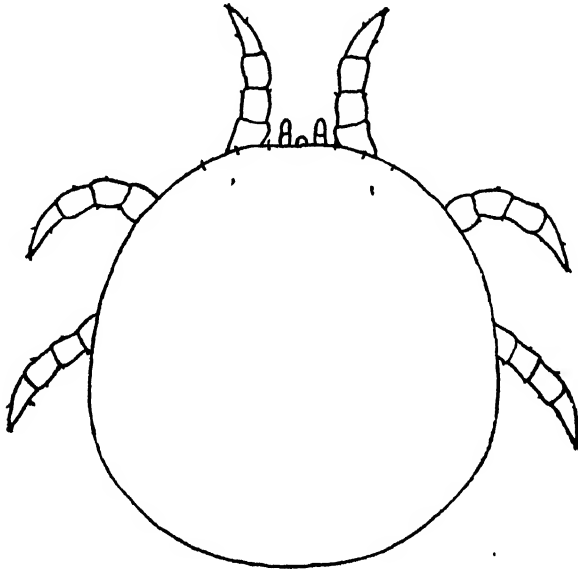
Fig. 33. *Argas moubata* Murray, dorsum of larva.
G. A. H. B. del.

GEOGRAPHICAL DISTRIBUTION.—The ticks are mainly found in native huts and in sand in desert-like localities. In the Transvaal specimens have been found at Lydenburg (Neumann), Acorn Hoek and Onderstepoort. In the Cape Province at Vryburg and other localities. It has also been recorded from Koffiefontein in the Orange Free State (Neumann), Natal (Howard), Bechuanaland, South-west Africa, Angola, Northern and Southern Rhodesia, Belgian Congo, French Congo, Cameroons, Kenya Colony, Uganda, Nyasaland, Tanganyika Territory, Zanzibar, Sudan (King), Abyssinia and Somaliland.

LIFE-CYCLE.—The life-history has been studied by Dutton and Todd (1905), Rodhain (1919), Cunliffe (1921) and Jobling (1924), and the process of copulation by Nuttall and Merriman (1911). The females lay their eggs in batches of from 28 or less to 340 after each feed, the greatest number being laid after the first and second meals, and the total number of eggs laid by a single female varies from about 44 to 1,217. The egg stage lasts 8 to 25 days, and the larvae on hatching are unable to feed. It has frequently been stated that they remain within the egg-shell, and differ from those of *A. savignyi* in this respect. However, both Jobling and myself have noticed that the larvae frequently free themselves from the egg-shell, and then become motionless and moult into nymphs 3 to 13 days later. After

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laying a batch of eggs, which takes on an average 7 days to accomplish, the female usually remains on them a few days longer until many have developed into their larvae or nymphs, and Jobling has noted that from 5 to 10 of the latter may usually be seen clinging to the ventral surface of the female, and may be transported by their parent some distance.

The male nymphs undergo 3 to 5 (usually 3) and the female nymphs 3 to 7 (usually 4) ecdyses before moulting into adults. Like the adults, they usually only attack their hosts at night and are ready to feed from 1 to 5 days after moulting, except the first stage nymphs, which require from 3 to 12 days. They feed but once between each ecdysis, taking from 13 to 87 minutes to gorge, those of the first stage and the stage preceding the adult usually taking the longest time to feed. The time they take to moult between each stage depends upon the preceding meal and not upon the preceding ecdysis. The shortest period a nymphal stage may last varies from about 5 to 9 days at 30° C. and from 9 to 13 days at 22° C.
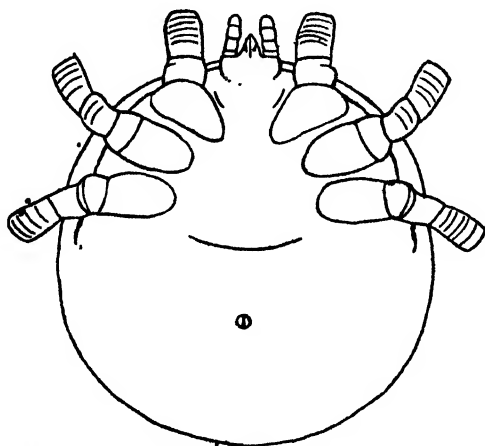
Fig. 34. *Argas moubata* Murray, venter of larva.
G. A. H. B. del.

The ratio of males to females is practically equal. Thus from one batch of 163 eggs, 72 males and 67 females were hatched (Jobling). The males are capable of fertilising the females before feeding and a single male may fertilise ten females, three before and seven after feeding (Jobling). In the males the interval beween the last ecdysis and feeding is much longer than in the case of nymphs and females, the interval being from 1 to 5 days in the latter. The females can be fertilised immediately after the last ecdysis, also after they have laid several batches of eggs. Jobling also observed a female that was fertilised four or more times in succession. The females usually commence egg-laying about 8 days after feeding and fertilisation. They take from 21 to 92 minutes to feed. Cunliffe observed the minimum periods required for a tick to undergo metamorphosis from egg to adult are as follows :—

Moults		4	5	6		5	6	7
At 22° C.	♂	64	87	104	♀	84	103	
At 30° C.		36	46	57		45	55	72

LONGEVITY.—Cunliffe kept a female performing normal functions alive for 862 days. The following were the longest periods he kept unfed ticks alive without food: A female for 105 days, a male for 244 days and a first stage nymph for 418 days.

TRANSMITTERS OF DISEASE.

RELAPSING FEVER OF TROPICAL AFRICA.—Although Livingstone, in 1857, suggested that tampans were responsible for the transmission of this disease in man, it was not until 1905 that Dutton and Todd in the Congo, and later Robert Koch, in Tanganyika Territory, experimentally proved that *A. moubata* transmitted *Treponema duttoni* (Novy and Knapp). The former authors not only found spirochaetes in the blood of monkeys on which they had fed ticks collected from native huts, but also made the important discovery that the offspring of infected ticks were capable of infecting animals on which they fed. Möllers (1907) has since shown that infected ticks do not lose the infection after feeding on six clean animals in succession. Furthermore, that the infection may be carried through to the third generation, even though the parents of these ticks had never fed on an infected animal.

Schuberg and Manteufel (1910) discovered that certain individuals of *A. moubata* may acquire an active immunity against infection with *T. duttoni*, and also *T. recurrentis*, and Hindle (1911a) found that about 30 per cent. of *A. moubata* from Uganda were immune to infection with *T. duttoni*.

Leichman (1909, 1910) studied the life-cycle of *T. duttoni* in the tick, and brought forward considerable evidence to show that at ordinary temperatures the salivary glands of the tick are not infected. This was confirmed by Hindle (1911a), who found that when ticks are kept at about 21° C. they harbour the parasites in the gut + contents, sexual organs, malpighian tubes and excrement, but not in the salivary glands and coxal fluid. When infected ticks were kept at a temperature of 35° C. for two or three days, the spirochaetes appeared in all the organs, and also in the coelomic fluid. The infection that may follow the bite of an infected tick would appear, therefore, to result from the entrance of the infective material, excreted by the tick whilst feeding, into the open wound caused by the tick's mouthparts.

The incubation period in man after an infected tick has bitten is usually 5 to 11 days, but may last 19 days.

The disease has been reported to occur along the east of Africa from Abyssinia in the north to Zululand in the south; also from Angola, Congo Free State and Northern Rhodesia. The relapsing fever reported from Zululand (Hindle, 1911b) is probably the same disease, especially as *A. moubata* occurs in this country.

Neumann (1909) demonstrated that *T. duttoni* could occasionally be transmitted from rat to rat by means of the rat louse, *Polyplax* (=*Haematopinus*) *spinulosa* (Burm.).

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OTHER RELAPSING FEVERS.—Manteufel (1909) showed that *A. moubata* could transmit *Treponema recurrentis* (Lebert), and Neumann (1909) transmitted *T. recurrentis*, *T. novyi* (Schellack) and also *T. duttoni* by means of this tick. Brumpt (1908b), however, failed to transmit both the Algerian and American relapsing fevers with this species.

FOWL SPIROCHAETOSIS.—Fülleborn and Mayer (1908) and Brumpt (1908b) demonstrated that *A. moubata* could transmit *Treponema anserinum* Sakharoff (= *T. gallinarum* Marchoux and Salimbeni) to fowls.

AEGYPTIANELLA PULLORUM CARPANO IN FOWLS.—Bedford and Coles (1933) failed to transmit the disease by feeding four adults of *A. moubata* on healthy chickens after they had fed on infected birds, and likewise failed by feeding nymphs, whose mother had previously fed on an infected fowl, on a healthy chicken.

DIPETALONEMA PERSTANS IN MAN.—Both Christy (1903) and Feldmann (1905) considered that *A. moubata* is capable of transmitting *Dipetalonema* (= *Filaria*) *perstans* (Manson) to man and Wellman (1907) observed a certain degree of development of *D. perstans* embryos in *moubata* raised from the egg. It is not known, however, to occur in the Union.

9. **Argas savignyi** Audouin.
" The Eyed Tampan Tick ".

Argas savignyi Audouin, 1827, *Des Égypte*, ed. 2, XXII, p. 426, pl. 9, f. 5.

Ornithodoros savignyi (Aud.) C. L. Koch, 1844, *Arch. Naturg.*, X, i, p. 219.

?*Ornithodoros morbillosus* Gerstaecker, 1873, *Decken's Reis, O.-Afr.*, III, ii, p. 464.

Argas schinzii Berlese, 1889, *Atti Soc. Veneto-Trent.*, X, p. 289, pl. 7, f. 5, 6.

Ornithodoros savignyi (Aud.), Nutt., Warb., Cooper and Robins., 1908, *Ticks: Mon. Ixod.*, i, p. 42, t.f. 58, 59-65, 70, 71 and pl. 2.

Ornithodoros savignyi (Aud.) Howard, 1908, *Ann. Tvl. Mus.*, I, ii, p. 83.

Ornithodoros savignyi (Aud.) Neumann, 1911, *Das Tierreich, Ixod.*, p. 123, f. 68, 69.

Ornithodoros savignyi (Aud.) Patton and Cragg, 1913, *A Textb. of Med. Ent.*, p. 586, pl. 72, f. 4, 5; pl. 75, f. 2, 3; pl. 83, f. 4-5; pl. 85, f. 7, 9.

Argas savignyi (Aud.) Bedford, 1932, *Rep. Dir. Vet. Serv. and Anim. Indust., Un. S. Afr.*, XVIII, p. 282, f. 7.

The *adults* (Fig. 3) and *nymphs* differ from those of *A. moubata* in possessing eyes, there being one pair on each side on the supracoxal folds.

HOSTS.—It is chiefly parasitic on man, and Lounsbury (1899) has recorded it from dog, horse, cattle, goat, pig and fowls. Patton and Cragg (1913) state that it feeds on camels, and is common along the camel caravan tracks in the Aden Hinterland. It also feeds on sheep (Howard, 1908) and rabbits.

GEOGRAPHICAL DISTRIBUTION.—This species is mainly found in loose soil in the shade of trees and rocks in desert-like localities; probably also in native huts. It is widely distributed in Africa and also occurs in parts of Asia. Lounsbury has recorded it from the Cape Province, Bechuanaland, Transvaal, Rhodesia and Portuguese East Africa. Two adults were found by the Verney-Lang Expedition to the Kalahari on sandy ground at Damara Pan, 1930. It has also been recorded from South-west Africa, Tanganyika Territory, Egypt (type locality), Sudan (King), Somaliland, Abyssinia, Nubia, Aden, Arabia and India.

LIFE-CYCLE.—The life history, which is similar to that of *A. moubata*, has been studied by Patton and Cragg (1913) and Cunliffe (1922). The females lay their eggs in batches of from 4 to 174 after each feed, and the total number of eggs laid by a single female varies from 100 to 417. The egg-stage lasts 7 to 28 days at 30° C., and the larvae on hatching are unable to feed. They usually free themselves from the egg-shell, but a small number may remain within the shells; they moult into nymphs after remaining motionless for 4 to 9 days. The male nymphs undergo 3 to 5 (usually 4) and the female nymphs 4 to 6 (usually 5) ecdyses before moulting into adults. Like the adults they usually only attack their hosts at night, and, as a rule, they feed but once between each ecdysis, taking from 10 to 74 minutes to gorge. The time they take to moult between each stage depends, as in the case with *A. moubata*, upon the preceding meal and not upon the preceding ecdysis. The shortest period a nymph stage may last varies from 8 to 15 days at 30° C. (Cunliffe). According to this observer the minimum periods required for a tick to undergo metamorphosis from egg to adult are as follws :—

Moults		4	5	6		5	6	7
At 30° C.	♂	60	73	89	♀	73	88	103

LONGEVITY.—Cunliffe kept a female performing normal functions alive at 30° C. for 420 days, and three females under similar conditions at 22° C. had an average life of 775 days.

TRANSMITTER OF DISEASE.—Brumpt (1908b) proved by experiments on animals that this species can transmit a spirochaete derived from cases of human relapsing fever occurring in Abyssinia.

10. **Argas pavimentosus** (Neumann).

Ornithodoros pavimentosus Neumann, 1901, *Mém. Soc. Zool. France,* XIV, p. 257.

Ornithodoros pavimentosus (Neu.) Dönitz, 1906, *Sitz.-Ber. Ges. naturf. Freunde,* p. 145, f. 2, 3.

Ornithodoros pavimentosus (Neu.) Nutt., Warb., Cooper and Robins., 1908. *Ticks; Mon. Ixod.,* i. p. 62, f. 90-92.

Ornithodoros savignyi var. *pavimentosus* (Neu.) Howard, 1908, *Ann. Tvl. Mus.,* I, ii, p. 87, pl. 3, figs. f, h.

Ornithodorus pavimentosus (Neu.) Dönitz, 1910, *Die Zecken Südafr.,* p. 413, pl. 16A, f. 8, 9.

Argas pavimentosus (Neu.) Bedford, 1932, *Rep. Dir. Vet Serv. and Anim. Indust., Un. S. Afr.,* XVIII, p. 281.

This species differs from *A. savignyi* in having the body covered with contiguous flat granulations which are smaller on the depressed areas, and the protarsi and tarsi of legs i, ii and iii are much shorter with the dorsal protuberances arranged closer together. Coarse setae present on body, especially numerous anteriorly.

Described by Neumann from a single female collected at Bethany, South-west Africa, and by Dönitz from numerous specimens collected by Schultze, also in Namaqualand. Schultze reported that they were common in places where travellers rest, attacking men when they lie upon the ground. Howard records receiving numerous specimens from South-west Africa, and states that the life history of the tick resembles that of *moubata* (= *caecus*) in the main.

Family *IXODIDAE*.

Key to the Genera.

1. Integument of body leathery, having a definite pattern and resembling that of Argasidae; scutum resembling the rest of body-integument, especially parts thereof; palpi short, the joints flexible, the third and fourth cylindrical, the latter being terminal; eyes absent; anal groove curving in front of anus ... ... *Nuttalliella* Bedford, p. 95
   Integument of body without a definite pattern; scutum not resembling rest of body-integument; palpi long or short, joints not flexible, the fourth situated ventrally at the distal end of the third segment 2

2. Anal grooves surrounding the anus in front or circular 3
   Anal grooves surrounding the anus behind (in *Boophilus* and *Margaropus* the anal groove is faint or obsolete) 4

3. Inornate, eyes and festoons absent; males with a pregenital, median, anal, two adanal and two epimeral plates on the venter. ... ... *Ixodes* Latr.

4. Hypostome and palpi short 5
   Hypostome and palpi long 10

5. Eyes absent ... ... *Haemaphysalis* Koch.
   Eyes present 6

6. Festoons present 7
   Festoons absent 9

7. Males with coxae iv much larger than coxae i to iii, no plates or shields on ventral surface of male 8
   Males with coxae iv not larger than coxae i to iii, a pair of adanal shields and usually a pair of accessory adanal shields on ventral surface of male. Species usually inornate, basis capituli generally hexagonal dorsally. *Rhipicephalus* Koch.

8. Species ornate, basis capituli rectangular dorsally.
   Dermacentor Koch.
   Species inornate, basis capituli hexagonal dorsally with prominent lateral angles. Coxae iv of male with two long spines... ... ... *Rhipicentor* Nutt. and Warb.

9. Inornate; coxae i with a small spine. Male with median plate projecting backwards on either side of the anus, and with a caudal protrusion when engorged. Fourth pair of legs of male dilated ... ... *Margaropus* Karsch.
Inornate; coxae i bifid. Male with a pair of adanal and accessory shields, and a caudal protrusion. Fourth pair of legs normal ... ... *Boophilus* Curtis.

10. Eyes present 11
Eyes absent or rudimentary 12

11. Festoons absent or present. Males with a pair of adanal shields and two posterior abdominal protrusions, accessory adanal shields absent or present ... ... *Hyalomma* Koch.
Species usually ornate; festoons present. Male without adanal shields, but small plaques may be present on the venter near the festoons ... ... *Amblyomma* Koch.

12. Species occurring almost exclusively on Reptilia.
Aponomma Neumann.

As the larvae do not always exhibit generic characters, the following key will serve to defferentiate the known South African species : —

Key to the known South African Larvae.

1. Eyes absent 2
Eyes present ... ... ' 8

2. Palpi long and slender 3
Palpi short, salient laterally 6

3. Scapulae not projecting forwards; anal groove, if present, surrounding the anus in front 4
Scapulae prominent, projecting forwards; anal groove contouring the anus behind; coxae i-iii each with a well-developed spur ... ... *Aponomma exornatum* Koch.

4. Basis capituli with lateral margins sub-parallel.
Ixodes simplex Neu., f. 36.
Basis capituli with lateral margins otherwise 5

5. Hypostome with 10 outer teeth on each side, and 3 rows of 3/3; palpi very long ... ... *Ixodes ugandanus* Neu., f. 37.
Hypostome with 8 outer teeth on each side, and 2 rows of 3/3; palpi shorter ... ... *Ixodes pilosus* Koch, f. 35.

6. First joint of palpi with a retrograde process on venter, basis capituli without cornua; coxae unarmed, rounded posteriorly ... ... *Haemaphysalis leachii* (Aud.) f. 38
First point of palpi without a retrograde process; coxae ii-iii unarmed 7

7. Basis capituli without cornua; coxa i with a pointed internal spur ... ... *Haemaphysalis parmata* Neu. (f. 39).
Basis capituli with cornua; coxa i with a slight blunt spur.
Haemaphysalis hoodi Warb. and Nutt. (f. 41).

8. Palpi and hypostome short 9
Palpi long and slender; hypostome long 16

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9. Basis capituli pointed laterally; scapulae with prominence on inner margin **10**

Basis capituli with lateral margins rounded **12**

10. Basis capituli with very acute lateral angles.
Rhipicephalus simus Koch (f. 44).

Basis capituli with lateral angles less acute **11**

11. Scutum more than twice as long as broad, with posterior margin only slightly convex.
Rhipicephalus sanguineus (Latr.) (f. 45).

Scutum less than twice as long as broad, with posterior margin considerably more convex.
Rhipicephalus appendiculatus Neu. (f. 43).

12. Scutum almost as long as broad; scapulae with prominence on inner margin ... ... *Rhipicephalus evertsi* Neu. (f. 42).

Scutum considerably wider than long; scapulae without prominence on inner margin **13**

13. Palpi very broad; scutum almost twice as wide as long, with posterior margin semicircular.
Margaropus winthemi Karsch (f. 46).

Palpi narrower, especially at apex; scutum longer in proportion to width **14**

14. Palpi shaped as in fig.
Rhipicentor nuttalli Cooper and Robins. (f. 40).

Palpi shaped as in figs **15**

15. Posterior margin of scutum semi-circular.
Boophilus microplus (Can.) f. 48).

Posterior margin of scutum more sharply rounded.
Boophilus decoloratus (Koch) (f. 47).

16. Scutum 0.28×0.37 mm. with posterior margin rounded.
Hyalomma aegyptium impressum Neu. (f. 49).

Scutum wider, 0.26×0.46 mm., with posterior margin more pointed ... ...: ... ... *Amblyomma hebracum* Koch (f. 50).
Amblyomma variegatum (Fabr.).
Amblyomma nuttalli Dönitz.
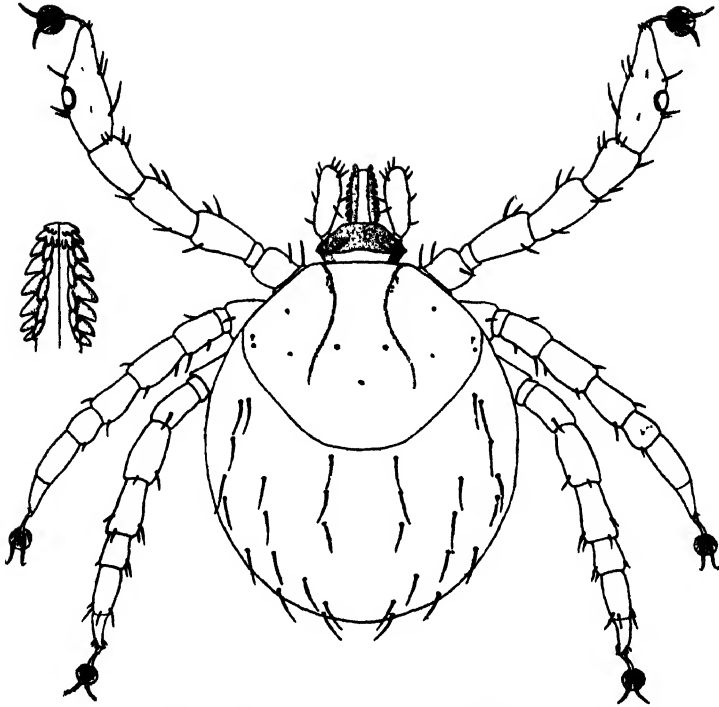
Fig. 35. *Ixodes pilosus* Koch, dorsum of unfed larva and hypostome.
G. A. H. B. del.
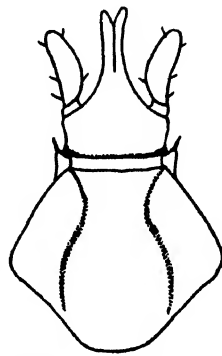
Fig. 36. *Ixodes simplex* Neu., capitulum and scutum of larva.
G. A. H. B. del.

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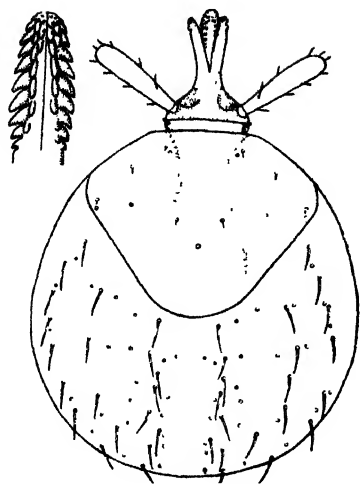
Fig. 37.   Fig. 38.

Fig. 37. *Ixodes ugandanus* Neu., dorsum of unfed larva and hypostome.
G. A. H. B. del.
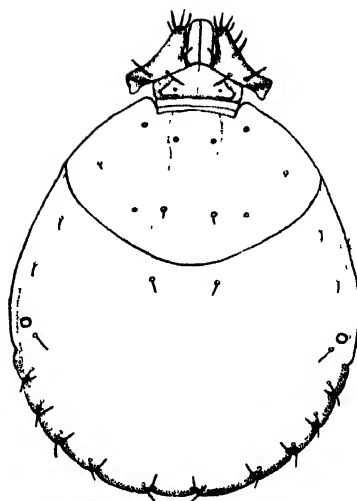
Fig. 38. *Haemaphysalis leachii* (Aud.), dorsum of unfed larva.
G. A. H. B. del.
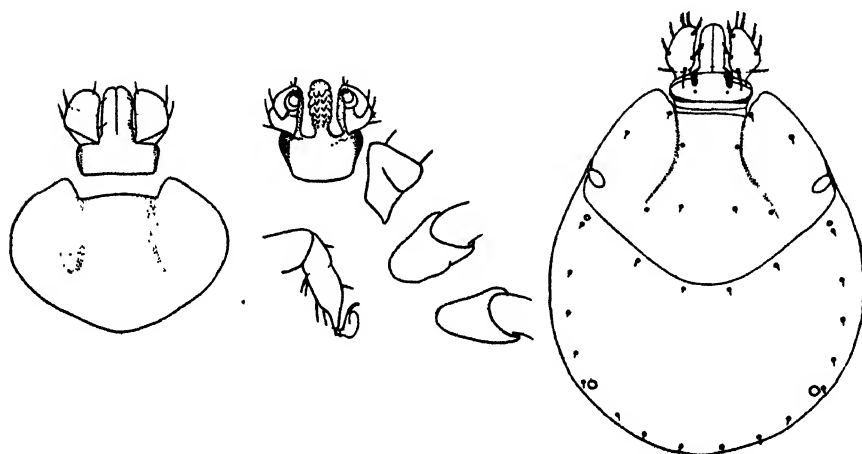
Fig. 39.   Fig. 40.

Fig. 39. *Haemaphysalis parmata* Neu., Larva. Scutum, capitulum in dorsal and ventral aspect, coxae and tarsus iii. (After Nuttall and Warburton.)

Fig. 40. *Rhipicentor nuttalli* Cooper and Robins, dorsum of unfed larva.
G. A. H. B. del.
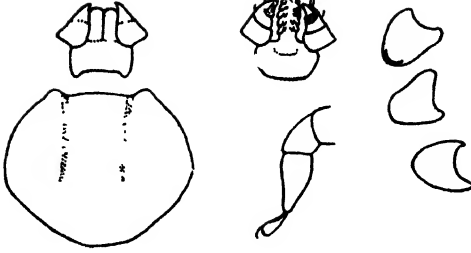
Fig. 41.   Fig. 42.

Fig. 41. *Haemaphysalis hoodi* Warb. and Nutt., larva, scutum, capitulum in dorsal and ventral aspect, coxae and tarsus iii. (After Nuttal and Warburton.)
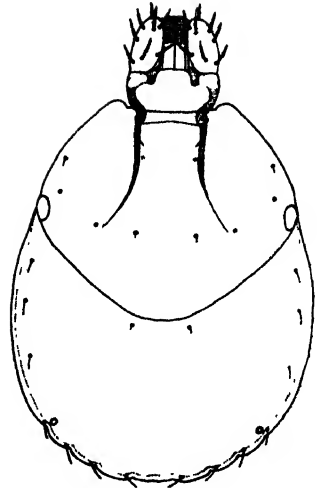
Fig. 42. *Rhipicephalus evertsi* Neu., dorsum of unfed larva.
G. A. H. B. del.
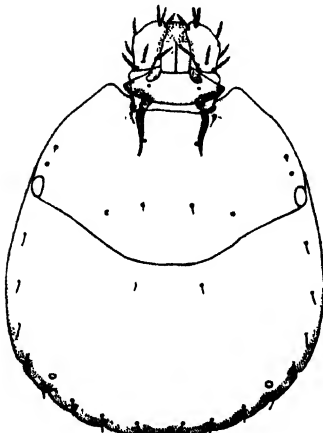
Fig. 43.   Fig. 44.

Fig. 43. *Rhipicephalus appendiculatus* Neu., dorsum of unfed larva.
G. A. H. B. del.
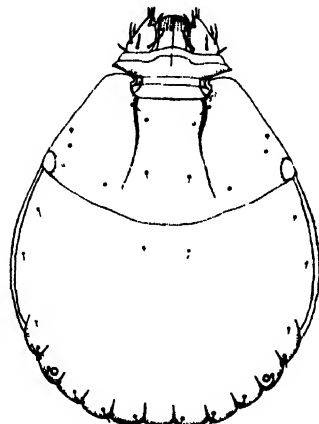
Fig. 44. *Rhipicephalus simus* Koch, dorsum of unfed larva.
G. A. H. B. del.
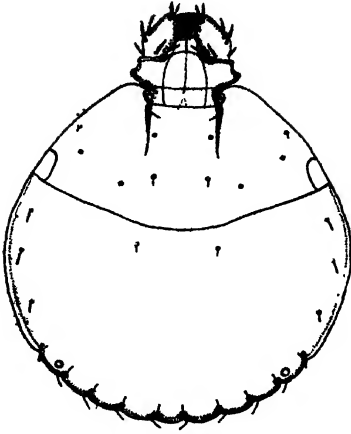
Fig. 45.

Fig. 46.

Fig. 45. *Rhipicephalus sanguineus* (Latr.), dorsum of unfed larva. G. A. H. B. del.
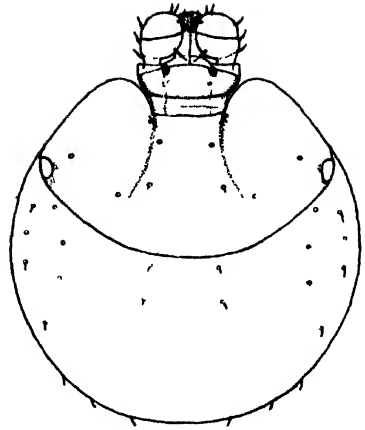
Fig. 46. *Margaropus winthemi* Karsch, dorsum of unfed larva. G. A. H. B. del.

Fog. 47.
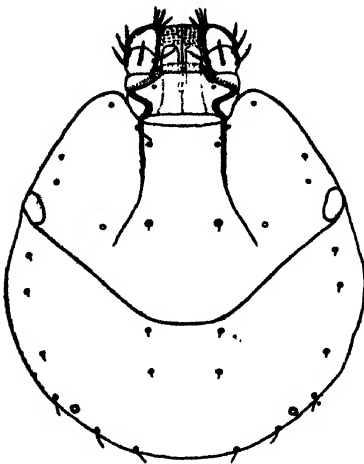
Fig. 48.

Fig. 47. *Boophilus decoloratus* (Koch), dorsum of unfed larva. G. A. H. B. del.
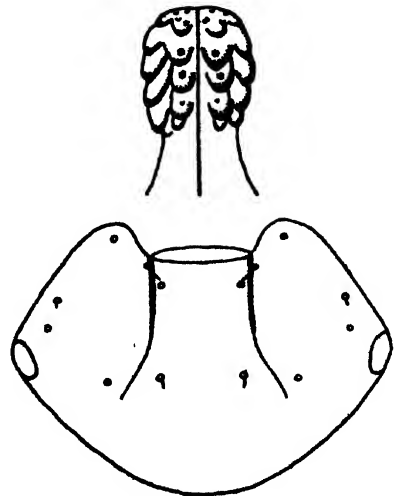
Fig. 48. *Boophilus microplus* (Can.), larva, scutum and hypostome. G. A. H. B. del.
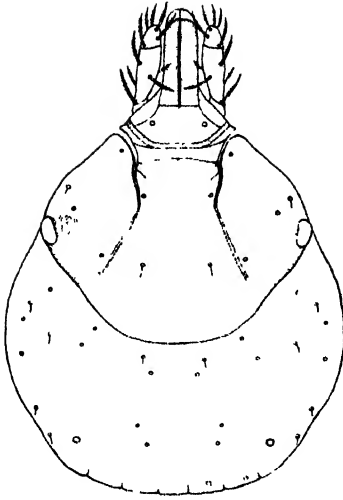
Fig. 49.   Fig. 50.

Fig. 49. *Hyalomma aegyptium impressum* Neu., dorsum of unfed larva.
G. A. H. B. del.
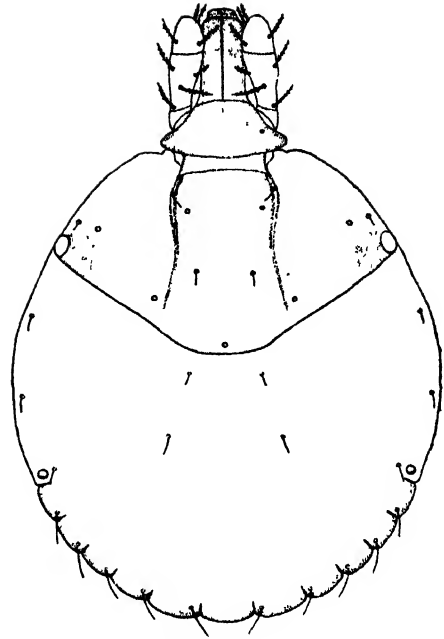
Fig. 50. *Amblyomma hebraeum* Koch, dorsum of unfed larva.
G. A. H. B. del.

Genus NUTTALLIELLA Bedford.

Nuttalliella Bedford, 1931, *Parasit.*, XXIII, ii, p. 231.

FEMALE CHARACTERS.—*Integument of body* leathery, having a definite pattern (see figs. 52, 53) and resembling that of Argasidae, more especially certain *Argas* spp. *Scutum* somewhat resembling the rest of the body-integument, especially parts thereof. *Eyes* absent. *Capitulum* situated on anterior margin; porose areas absent. *Hypostome* very short. *Chelicerae* present. *Palpi* short, the joints very flexible; basal segment very small, the second much the largest and grooved on inner surface; the third and fourth cylindrical, the latter terminal. *Anal groove* curving in front of anus. *Genital* and *dorsal grooves* absent. *Festoons* absent. *Legs* with most of the joints incised apically on the ventral side; coxae i and ii situated close together; coxae ii, iii and iv widely separated; tarsi without spurs. Haller's organ present on tarsus i.

This genus contains a single species, *Nuttalliella namaqua* Bedford.

Nuttalliella namaque Bedford.

Nuttalliella namaqua Bedford, 1931, *Parasit.*, XXIII, ii, p. 231,
     text fig. 1, pl. 10, f. 1, 2.

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ENGORGED FEMALE (Figs. 51-53) (f. 50).—Body slate-coloured, slightly wider behind than in front, $4 \times 3 \cdot 5$ mm., integument pitted (fig. 51), the pits being very shallow and more or less equidistant apart. *Capitulum* (fig 51 A.B.) orange-coloured with base very short dorsally, elongated ventrally ($0 \cdot 42 \times 0 \cdot 27$ mm.), having the lateral margins parallel, the anterior margin straight and the posterior margin slightly convex. *Hypostome* with one or two very rudimentary teeth. *Scutum* (fig. 51A) considerably wider than long ($0 \cdot 57 \times 1 \cdot 07$ mm.), with two large very deep depressions behind, one
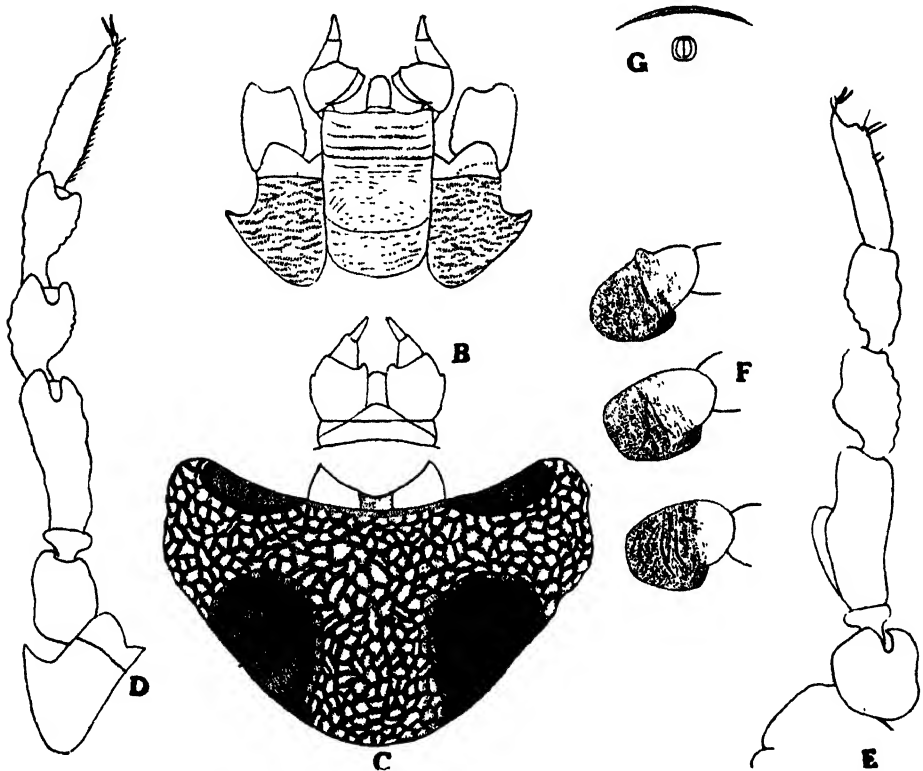
Fig. 51. *Nuttalliella namaqua* Bedford. A. Capitulum and coxae i in ventral aspect. B. Capitulum in dorsal aspect. C. Scutum. D. First leg, ventral aspect. E. First leg, lateral aspect. F. Coxae ii-iv, drawn separately. G. Anus with crescentic pre-anal groove.
G. A. H. B. del.

on each side on the posterior margin, and two smaller ones on the anterior margin; these depressions are dark and closely resemble the rest of the integument of the body, so much so that it is difficult to see the dividing line between them; the rest of the scutum is pale in colour and honeycombed with deep irregular pits. It is situated well forward on the anterior margin, lies almost at right angles to the capitulum, and can, therefore, hardly be seen when the tick is viewed from above. In an unengorged specimen it probably lies in the same or almost the same plane as the capitulum. *Legs* orange-
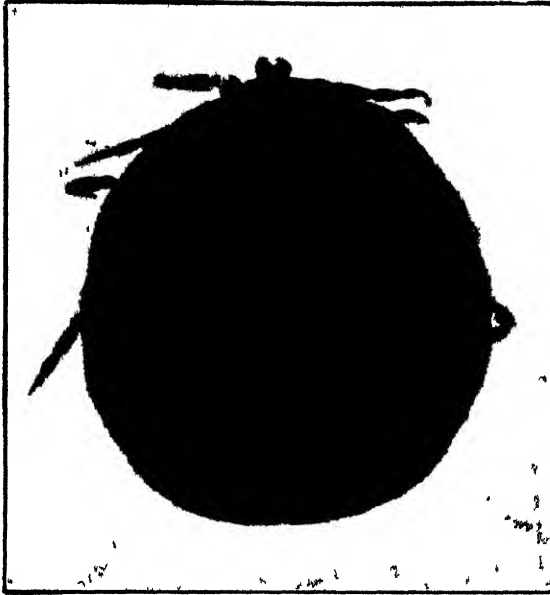
Fig. 52. *Nuttalliella namaqua* Bedford, dorsum of engorged female.
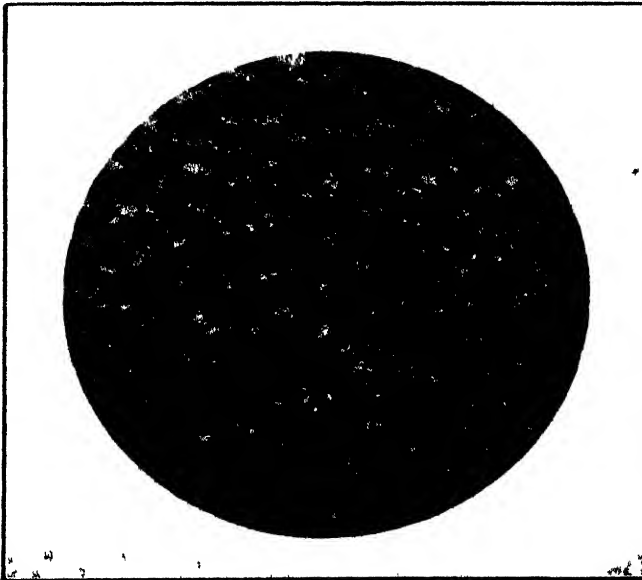
Fig. 53. *Nuttalliella namaqua* Bedford, portion of body integument highly magnified.

coloured, pale at the apices of the joints, except coxae ii-iv, which are dark basally and pale apically; coxa i with a large spur on its outer margin; coxa ii with a small spur on its anterior margin; coxae iii and iv unarmed, the remainder of the joints of legs ii to iv very similar to those of the forelegs, which are shown in figs. 51, D and E. *Genital opening* situated between coxae ii. Anus clothed with numerous minute setae, and situated a short distance in front of the posterior margin. *Anal groove* (fig. 51 G) rudimentary, pre-anal, and does not continue backwards towards the posterior margin.

The type is deposited in the South African Museum, Capetown. This species was described from an engorged female collected under a stone at Kamieskroon, Little Namaqualand, by Dr. R. F. Lawrence in October, 1930. Dr. Lawrence informs me that rock-rabbits were probably the commonest animals about the hill where he found it, but the host of the tick may be a bird.

LIST OF REFERENCES.

References to the literature on ticks will be given at the end of the final part.

Spirochaetosis in Man and Poultry.

BRUMPT, E. (1908a). Existence de la " fièvre des tiques " en Abyssinie. Quelques mots sur la biologie de l'*Ornithodorus moubata*, acarien qui détermine cette spirochétose. *Bull. Soc. Path. Exot.*, Vol. 1, pp. 432-436.

BRUMPT, E. 1908b). Transmission du *Spirochaeta duttoni* par l'*Ornithodorus savignyi*. Transmission du *Spirochaeta duttoni* et du *Spirochaeta gallinarum* par l'*Ornithodorus moubata*, etc. *Ibid.*, pp. 578-581.

BRUMPT, E. (1913). Précis de Parasitologie. 2nd Edition, Paris.

BRUMPT, E. ET FOLEY (1908). Existence d'une spirochetose des poules à *Spirochaeta gallinarum* R. Bl. dans le Sud-Oranais. Transmission de cette maladie par *Argas persicus* *C.R. Soc. Biol.*, Vol. 65, p. 132.

CASTELLANI, A. AND CHALMERS, A. J. (1910). Manual of Tropical Medicine. 1st edition, London.

DUTTON, J. E. AND TODD, J. L. (1905). The nature of human tick fever in the eastern part of the Congo Free State. *Brit. Med. Journ.*, Vol. 2, p. 1259; also Liverpool Sch. Trop. Med., Mem. 17.

FÜLLEBORN UND MAYER, M. (1908). Über die Möglichkeit der Übertragung pathogenen Spirochaeten durch verscheidene Zeckenarten. *Arch. Schiffs.-u. Trop. Hyg.*, Vol. 12, p. 31.

HINDLE, E. (1911a). The Transmission of *Spirochaeta duttoni*. *Parasit.*, Vol. 4, pp. 133-149.

HINDLE, E. (1911b). The Relapsing Fever of Tropical Africa. A Review. *Ibid.*, Vol. 4, pp. 183-203, 2 maps and 1 chart.

HINDLE, E. (1911c). On the Life-cycle of *Spirochaeta gallinarum*. Preliminary Note. *Ibid.*, Vol. 4, pp. 463-477, f. 1-5 and 1 diagram.

HINDLE, E. (1912). The Inheritance of Spirochaetal Infection in *Argas persicus*. *Proc. Cambridge Philos. Soc.*, Vol. 16, p. 457.

KLEINE, F. K. UND ECKARD, B. (1913). Ueber die Lokalisation der Spirochäten in der Rückfallfieberzecke (*Ornithodorus moubata*). *Zeitschr. Hyg. u. Infekt.*, Vol. 74, p. 389.

KNUTH, P. UND DU TOIT, P. J. (1921). Die Spirochätosen. *Mense's Hand. d. Trop. Krankh.*, Liepzig.,

KOCH, R. (1905). Vorläufige Mitteilungen über die Ergebnisse einer Forschungsreise nach Ostafrika. *Deutsch med. Wochenschr.*, p. 1866.

LEICHMANN, W. B. (1909). Preliminary note on experiments in connection with the Transmission of Tick Fever. *Jnl. R.A. Med. Corps.*, Vol. 12, pp. 123-135.

LEICHMANN, W. B. (1910). An address on the Mechanism of Infection in "Tick Fever" and on the Hereditary Transmission of *Spirochaeta duttoni* in the Tick. *Lancet*, Vol. 178, pp. 11-14.

LIVINGSTONE (1857). Missionary Travels and Researches in South Africa. John Murray, London, pp. 628-629.

MANTEUFEL (1909). Weitere Untersuchungen über Rückfallfieber. *Arb. a.d. Kais. Gesundheitsamte*, Vol. 29, pp. 337-354.

MARCHOUX, E. et SALIMBENI, A. (1903). La spirillose des poules. *Ann Inst. Pasteur.*, Vol. 17, p. 569.

MÖLLERS, B. (1907). Experimentelle Studien über die Uebertragung des Rückfallfiebers durch Zecken. *Zeitschr. f. Hyg. u. Infections-Krankh.*, Vol. 58, pp. 277-285.

NEUMANN, R. O. (1909). Ueber das Verhalten der Spirochäten des Rückfallfiebers im Tierkörper und die experimentelle Uebertragung der Parasiten durch Zecken und Läuse. (*Naturhist. med. Ver.*, *Heidelberg*, 12 Jan.) *München. med. Wochenschr.*, Vol. 56, p. 477.

NUTTALL, G. H. F. (1913). The Herter Lectures. 1 Spirochaetosis. *Parasit.*, Vol. 5, pp. 262-274.

SCHUBERG und MANTEUFEL (1910). Ueber erworbene Immunität gegen Rekurrens bei *Ornithodorus moubata*. *Zeitschr. f. Immunitätsforschung*, Vol. 4, pp. 512-515.

WENYON, C. M. (1926). Protozoology. Part II. London.

Aegyptianella pullorum Carpano in Fowls.

BEDFORD, G. A. H. and COLES, J. D. W. A. (1933). The Transmission of *Aegyptianella pullorum* Carpano to Fowls by means of Ticks belonging to the genus *Argas*. *O.Poort. Jl. Vet. Sc. and An. Ind.*, Vol. 1, No. 1, pp. 15-18.

Dipetalonema perstans in Man.

CHRISTY, C. (1903). "Tick Fever" in Man. (Including a note by R. 1. Pocock). *Brit. Med. Journ.*, Vol. 2, p. 653; also *Thomps. and Johnst. Labs. Rep.*, Vol. 5, pp. 187-189, pl. 15.

FELDMANN (1905a). Ueber *Filaria perstans* im Bezirk Bukoba. *Arch. f. Schiffs. u. Tropen-Hygiene*, Vol. 9 (2), pp. 62-65, 1 pl.

FELDMANN (1905b). On *Filaria perstans* in the Bukota District. (Trans. by T. S. Kerr). *Journ. Trop. Med.*, Vol. 8 (8), pp. 125-126.

WELLMAN, F. C. (1907). Preliminary notes on some bodies found in ticks—*Ornith. moubata* (Murray)--fed on blood containing embryos of *Filaria perstans* (Manson). *Brit. Med. Journ.*, Vol. 2, p. 142.

The Vegetable Diet Theory of *Glossina Pallidipes*.

By

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INTRODUCTION.

IN a paper recently read before a meeting of Durban Members of the Royal Society of Tropical Medicine and Hygiene by Mr. A. Davidson, it was stated that the tsetse fly, *G. pallidipes*, can live on the latex of plants. This worker specially mentions *Euphorbia tirucalli* and *Sarcostemmus riminale* as being two of the latex bearing plants upon which the fly feeds. He declares that it will take up sufficient nutritive fluid therefrom to support life and even to reproduce.

No experimental proof is given, though it appears that this worker attempts to correlate the flagellate infection found in the latex of Euphorbia species and other latex bearing plants (described by Lafont in 1909), with the trypanosome infection normally found in Glossinae, and their vertebrate hosts, the wild animals.

The present investigation was conducted at the Nagana Research Station in Zululand.

G. pallidipes was the insect material employed. The latex was derived from the plants *E. tirucalli* and *Sarcostemmus riminale*, the species referred to by Mr. Davidson. Flagellate infection was found in *E. tirucalli* but none in *S. riminale*. These two species occur in abundance throughout the habitat of *G. pallidipes* in Zululand. During the experiments the tsetses were given every opportunity under both normal and artificial conditions to ingest latex from both of these plants. It will be shown that the insects do not in nature attempt to feed upon this substance, and therefore any theory in regard to the trypanosome infection of tsetses being derived from plant flagellates is entirely without foundation.

EARLY INVESTIGATIONS.

The history of Glossinology is presented in an instructive manner by Hegh (1930). Entomologists were at first content to describe the few tsetses brought home by travellers. Wiedman (1830) established his genus Glossina without comment. Robineau-Desvoidy boldly added to his description of *G. palpalis* the remark that " the proboscis was innocuous " by which he perhaps meant that the fly did not suck blood.

Macquart embroidered this opinion. " It is probable ", he says, " that this fly does not live on animal blood like stable flies, but on the nectar of flowers. The two setae contained in the proboscis, and forming a sucking apparatus are so fine that one can hardly conceive how they might be able to pierce the skin; the weakness of this organ seems to be further shown by the modifcation of the palpi, which are lengthened and hollowed out into a sheath for the proboscis." Indeed, the proboscis of the tsetse fly does seem eminently adapted to the sucking of nectar, yet none of the species have been seen visiting flowers. The males of the Tabanidae naturally suck the nectar from flowers, and the females of mosquitoes will suck both blood and nectar so that by analogy it is perhaps not difficult to imagine that some of the Glossina species might likewise visit flowers.

David Livingstone (1857) focussed the attention of the scientific world upon the ravages of the tsetse, and since that time entomologists, veterinarians, medical men, protozoologists, botanists and many others, even colonial politicians have studied the behaviour of this insect.

Ecological Factors.

Doubtless every species of *Glossina* is associated with a particular type of country on account of the fly's special requirements in regard to floral conditions, shade, etc., either as an adult or as a pupa.

The species *G. pallidipes* with which we now deal belongs to that group of tsetse which can live through prolonged dry seasons without apparent suffering, and in consequence is found in regions clothed with one of the many types of open vegetation included under the general term " Savannah ".

Ecological conditions alone, however, are inadequate to account for the local abundance or scarcity of tsetses. Their feeding habits are of more importance, and because they thrive best and most numerously where animal hosts are most abundant, there appears to be a very close association between the tsetses and the wild animals. There can be no doubt that the insects are strictly haematophagous.

The Tsetses Reaction to Visual Impressions.

In Nature, Glossinae while beset with many hazards and contingencies manage to thrive, notwithstanding the apparent handicap of extremely slow reproduction. An observer cannot help being impressed by the apparently unobtrusive, yet deliberate manner in which a tsetse when attacking, darts towards a victim, and it has been established beyond doubt that the fly hunts by sight, reacting in a peculiar manner to visual impressions.

The most convincing evidence of this is supplied by the operations carried out by Harris and his staff at the Nagana Research Station where millions of tsetse (*G. pallidipes*) have been destroyed by the " Harris " fly trap. This trap is a visual impression one, and has no olfactory stimulus as bait. The flies are attracted to it by its conspicuousness and its resemblance to the bulk of an animal, and,

darting towards the object they dive into the shadow of the ventral surface. They are then attracted to the light showing through the opening in the platform on which the cage rests; making their way through this into the cage above, and are caught.

Digestive Anatomy of the Fly.

There are many features in the external and internal anatomy of the tsetse which show a high degree of specialization. The mouth parts and the digestive tract offer, perhaps the most striking peculiarities, and are of importance in connection with the rôle of the flies as vectors of the disease.

(a) In the proboscis the mandibles and maxillae, found in some species of haematophagous diptera, are absent. The labrum and labium in apposition form together a needle-like tube enclosing the long slender hypopharynx. The saliva from the salivary glands passes into the wound when the proboscis punctures the skin of the vertebrate, and prevents coagulation of the blood.

At rest, the proboscis is carried horizontally between the palpi which ensheathe it. When the insect is about to feed the proboscis is lowered vertically, the palpi retaining the horizontal position. The skin is pierced by the rapid movement of the labella situated at the tip of the labium, and the proboscis is thrust into the wound as far as its bulbous base will permit. When a suitable well of blood has been found, the proboscis is partially withdrawn, and the blood may be seen passing up the tube, the fly becoming engorged in a few seconds.

(b) The digestive tract and the complicated process of digestion have recently been studied in great detail by Wigglesworth (1930).

Mr. Davidson passes over this creditable work with the following remarks: " Some observers go so far as to assert that the digestive organs of tsetse are only capable of dealing with blood; but this is a rash statement, since a gut that can digest blood would almost certainly be able to digest any fluid which contains suitable proteins, such as milk and the latex of plants ".

Mr. Davidson gives no points of similarity between milk and the latex of plants, and the reader is left to surmise that the substances are similar. Whereas milk is essentially a blood product of mammals, latex is a storage product of the green cells of plants.

Wigglesworth discusses the physiology of digestion in the cockroach, and shows that the properties of the digestive enzymes are similar to those of vertebrates. Comparing the digestive system of the tsetse fly (Glossina) with that of the blue-bottle (Calliphora), he states that the adaptation to a diet of blood in the former is the loss of enzymes acting upon carbohydrates, while he notes a great increase in the activity of the proteolytic enzymes and the acquisition of factors affecting blood coagulation.

Wigglesworth also suggests that the so-called " symbionts " found in the mid gut might be contributing some accessory food factor which is lacking in the sterile diet of their host.

In this connection it may be mentioned that if the region of the gut in which these symbionts occur is cut the symbionts float out, and, to an inexperienced worker they may readily appear similar to starch grains seen in the latex of euphorbias. Mr. Davidson has referred freely to starch grains in the gut of tsetses.

TEMPERATURE IN REGARD TO FEEDING.

Harris (1930) has shown that the tsetse *G. pallidipes* is highly sensitive to warmth, and responds to this stimulus by probing even when the warmth is in the form of hot air. He has pointed out that those flies, which on alighting do not explore a cool object, whether that object resembles a quadruped or not, appear to be those which because not acutely hungry require some accessory stimulus such as warmth to cause them to probe. Flies which immediately probe appear to be those in which hunger has reached an acute stage.

From these facts it would seem that there should be some contrast in temperature between an object and the surrounding media to induce most flies to probe.

The following temperatures of objects represent the averages of five readings taken over half an hour. The temperature of the air and that of the surface mentioned were read at the same time.

Temperature of air.....	24·5° C.	Temperature of bark of Euphorbia....	25·5° C.
Temperature of air.....	25·6° C.	Temperature of green Asclepiad.......	24·5° C.
Temperature of air.....	25·6° C.	Temperature of man's arm...........	34·9° C.
Temperature of air.....	29·2° C.	Temperature of donkey's skin........	34·8° C.

The Table shows that the temperature of the surface of trees may be lower or slightly higher than that of the surrounding atmosphere. On the other hand the temperature of the surface of a mammal is generally much higher than that of the surrounding atmosphere.

For this reason in the following experiments, when attempting to induce flies to feed on latex bearing plants, it was found necessary to warm their naturally cool surfaces in order to induce the tsetse to probe gainst them.

LATEX IN NATURE.

Latex occurs in certain plants of the families *Euphorbiaceae*, *Asclepiadaceae* and others. It is a milky fluid, a storage product of the green cells and is contained in lactiferous tubes running longitudinally in the xylem. When the stem of a latex bearing plant is punctured the latex exudes. The only manner in which the tsetse could feed upon the latex therefore, is by puncturing a containing vessel with its proboscis.

Under the microscope, dumb-bell shaped starch grains are seen in plenty in the latex of euphorbias, but similar starch grains have not been found in the asclepiad.

Any fly which ingested latex should upon dissection and examination reveal either traces of latex, or the peculiar shaped starch grains, in the gut.

MATERIAL AND METHOD.

The tsetses for the experiments were captured flies taken from the " Harris " traps operating in the Umfolozi game reserve.

Groups of ten flies each were placed in glass battery jars covered with mosquito netting. All flies were given a preliminary feed of blood from a donkey to ensure that none were hungry and that all might start the experiments on a common hunger basis.

Subsequently each group of flies was given the opportunity to feed daily on the respective foods, blood or latex, while some were starved to act as a control.

In that the flies used in the experiments were captured flies, no definite age could be assigned to them, though none were young.

When giving the flies the opportunity to feed on the latex bearing plants, twigs were placed side by side and clamped at the ends to prevent movement, and to form a mat presenting a more or less even surface. The mat was placed over a basin of warm water (37° C. to 40° C.) and left for a few minutes in order to warm the twigs to give inducement for the flies to probe. Both plants, *S. rimmale* and *E. tirucalli* were employed.

The jar containing the flies was then inverted on the mat so that the mosquito net covering the jar came into contact with it. By this method it was possible for the proboscides of the flies to reach the twigs without difficulty. Similarly when feeding the flies on the donkey, the jar was inverted on the shaven skin of the animal.

When the flies died they were dissected, and the gut contents specially examined for traces of latex. The surface of the plants was also examined for signs of exudation.

The experiments were twice repeated, and the results are given in the following Tables.

FLIES FED ON BLOOD OF DONKEY.

Table I shows that of the ten flies in the jar eight lived fifteen days and two fourteen days. Their deaths were due to a succession of unusually hot days when the highest temperature recorded was 105° F., and the relative humidity dropped to 15 per cent. Seven of them deposited larvae of which six successfully emerged.

The experiment was repeated, the results being given in Table II. It is here seen that four flies died after seventeen and eighteen days, one after forty-two and another after forty-three days. The four remaining flies lived fifty-seven days, when the experiment was stopped. Eleven larvae were deposited, and there was one abortion. Flies emerged from all the puparia.

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TABLE I.

Flies Given Opportunity to Feed on Donkey.

Date.	No. of Flies.	Died.	Days Lived.	Larvae Deposited.	Emerged.
1/10/32	10	—	—	—	—
—	—	—	—	1, 6/10/32	12/11/32, ♀
—	—	—	—	1, 8/10/32	15/11/32, ♀
—	—	—	—	2. 9/10/32	16, 18/11/32, ♀, ♂
—	—	—	—	2, 11/10/32	18, 21/11/32, ♀, ♂
14/10/32	10	—	—	1, 15/10/32	—
15/10/32	8	2♀	14	—	—
16/10/32	—	3♂, 5♀	15	—	—

TABLE II.

Flies Given Opportunity to Feed on Donkey.

Date.	No. of Flies.	Died.	Days Lived.	Larvae Deposited.	Emerged.
21/10/32	10	—	—	—	—
—	—	—	—	1. 25/10/32	27/11/32, ♀
—	—	—	—	1, 26/10/32	1/12/32, ♂
—	—	—	—	1, 27/10/32	1/12/32, ♂
—	—	—	—	1, 1/11/32	3/12/32, ♀
—	—	—	—	1, 2/11/32	7/12/32, ♂
6/11/32	10	—	—	1. 4/11/32	11/12/32, ♂
7/11/32	7	1♂, 2 ♀	17	—	—
8/11/32	6	1♂	18	—	—
—	—	—	—	1, 17/11/32	21/12/32, ♂
2/12/32	5	1♂	42	1, 21/11/32	♂
3/12/32	4	1♂	43	1 Abortion,	—
—	—	—	—	27/11/32	—
—	—	—	—	1, 29/11/32	♀
—	—	—	—	1, 1/12/32	♂
16/12/32	—	1♂, 3♀	57	—	—

TABLE III.

Flies Given Opportunity to Feed on Latex-bearing Plant.

SARCOSTEMNUS VIMINALE.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
1/10/32	10	8	—	—	—	Fed on blood.
2/10/32	—	—	—	—	—	Stimulated to probe by warmth.
4/10/32	9	—	1♀	3	—	Thin.
5/10/32	9	—	—	—	1 Abortion	—
6/10/32	7	—	1♂, 1♀	5	—	—
7/10/32	5	—	1♂, 1♀	6	—	Starving.
8/10/32	0	—	1♂, 4♀	7	—	—

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TABLE IV.

Flies Given Opportunity to Feed on Latex-bearing Plant.

SARCOSTEMNUS VIMINALE.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
22/10/32	—	—	—	—	—	Stimulated to probe by warmth.
24/10/32	10	—	—	—	1 Abortion	—
25/10/32	10	—	—	—	1 Abortion	—
26/10/32	8	—	2♀	5	—	—
28/10/32	7	—	1♀	7	—	Flies weak.
29/10/32	3	—	3♂, 1♀	8	—	—
30/10/32	3	—	—	—	—	—
31/10/32	2	—	1♀	10	—	—
1/11/32	0	—	1♂, 1♀	11	—	—

TABLE V.

Flies Given Opportunity to Feed on Latex-bearing Plant.

EUPHORBIA TIRUCALLI.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
22/10/32	10	—	—	—	—	Stimulated to probe by warmth.
25/10/32	9	—	1♀	4	1 Abortion	—
26/10/32	6	—	2♂, 1♀	5	—	Thin.
28/10/32	4	—	1♂, 1♀	7	—	—
29/10/32	2	—	1♂, 1♀	8	—	—
31/10/32	1	—	1♀	10	—	—
1/11/32	0	—	1♂	11	—	—

FLIES GIVEN THE OPPORTUNITY TO FEED ON LATEX-BEARING PLANTS.

As will be seen in Table III eight flies had a preliminary feed of blood. On the following day the jar of flies was inverted on the mat of *S. viminale*, previously described. None attempted to feed. Next day the mat was again warmed over a basin of hot water and the flies given further opportunity to pierce the twigs. This procedure was repeated daily. On each occasion the flies reacted to the warmth of the mat, but were never at any time able to pierce the cuticle and epidermis of the twigs, and thus did not feed. As the days passed, the flies became more and more hungry, and the probing reaction to warmth became more pronounced. On the third and forth day they were definitely thin and hungry, and eventually became too weak to attempt to probe. The last flies died on the seventh day. One female aborted on the fifth day, but there were no births.

This experiment was repeated with another batch of twenty flies, when both of the latex bearing plants *S. viminale* and *E. tirucalli* were used.

Methods of feeding similar to the foregoing were employed. As seen in Table IV and V the longest lives recorded were eleven days. There were three abortions. When the flies reacted to the stimulus of warmth, none were able to pierce the twigs. The action of the proboscides against the twigs was carefully watched, and though numerous attempts were made to pierce them none succeeded. Microscopic examination of the surface of the mat revealed no signs of exuding latex, such as would occur had the cuticle been punctured.

All dead flies from the " latex jars " were dissected. The abdomens were totally collapsed, and the guts contained only greenish traces of digested blood at the posterior end. No trace of latex was found, and the iodine test for starch gave no reaction. In all cases the proboscides contained no trace of latex.

FLIES GIVEN THE OPPORTUNITY TO FEED ON LATEX THROUGH A MEMBRANE.

As the flies were unable to pierce the epidermis of the latex-bearing plants, it was decided, to tap the latex into tubes, to cover them with a membrane and endeavour to induce the flies to feed through it.

Two types of membrane were used. The skin of a freshly killed bird, and the caecum of an antelope. In the former case the skin, devoid of feathers, was stretched tightly over the mouth of the tube filled with latex. In the latter case the blind caecum was filled and tied at the open end.

The latex of both *Euphorbiaceae* and *Asclepiadaceae* tend to coagulate when drawn from the plant forming a viscous fluid. For this reason it was necessary to dilute the latex with physiological saline for the flies to ingest. A mixture of citrated blood and latex was also tried as a diet.

As in the case of the plant experiment it was necessary to warm the mixture slightly (37° C. to 39° C.) in order to stimulate the flies to probe.

Only by presenting the latex in this extremely artificial manner was it found possible to induce the flies to feed.

RESULTS.

Two jars of ten flies each were employed. They were first given a feed of blood to ensure working from a common hunger basis. They were then starved for three days so that all were sufficiently hungry to respond readily to the stimulus of warmth.

The jars containing the flies were inverted, and the membrane covering the tubes of warm latex was brought into contact with the flies. Stimulated by the warmth the tsetses probed and pierced the membrane, but rapidly withdrew the proboscis and commenced cleaning it with their front legs. In several cases the flies sucked up some of the latex and fell on their backs, dying in a few minutes, while in other cases they survived from one to twelve hours,

according to the amount of material ingested. Similar findings were noticed in the case of the latex of the Asclepiad and in the mixture of blood and latex.

On dissection of the dead flies the dumb-bell shaped starch grains of the Euphorbia were found in their guts, and the proboscides were clogged with latex.

FLIES GIVEN THE OPPORTUNITY TO FEED ON CITRATED BLOOD THROUGH A MEMBRANE.

To ensure that the above method of feeding flies through a membrane could have no adverse effect upon the length of life of the fly, a jar of twelve flies was fed experimentally upon citrated blood alone.

The blood was drawn from a donkey into tubes containing a 2 per cent. solution of potassium citrate, the mixture comprising 12 c.c. of blood and 2 c.c. of citrate.

As previously the skin of a bird was stretched tightly over the mouth of the tube and it was then placed in a water bath and kept at a temperature between 37° C. and 39° C.

The tube containing the warm citrated blood was then brought into contact with the tsetses.

Stimulated by the warmth the flies pierced the membrane and their abdomens swelled in a few seconds, as though engorging on an animal host.

Table VI shows that the flies were given the opportunity to feed in this manner on nine occasions. The opportunities to feed varied from one to five days interval.

TABLE VI.

Flies Given Opportunity to Feed Through a Membrane on Citrated Blood.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae. Deposited.	Remarks.
15/6/33	12	12	—	—	—	Fed through a membrane.
16/6/33	12	—	—	—	—	—
17/6/33	11	6	1♀	2	3 Abortions..	Fed through a membrane.
19/6/33	11	—	—	—	1 Pupa......	—
20/6/33	11	—	—	—	2 Abortions..	—
21/6/33	9	9	2♀♀	6	1 Pupa......	Fed through a membrane.
24/6/33	9	7	—	—	—	Fed through a membrane.
27/6/33	9	9	—	—	1 Pupa......	Fed through a membrane.
1/7/33	9	7	—	—	—	Fed through a membrane.
3/7/33	9	—	—	—	1 Pupa......	—
5/7/33	8	8	1♂	20	—	Fed through a membrane.
11/7/33	8	5	—	—	—	Fed through a membrane.
12/7/33	6	—	2♀♀	27	—	—
15/7/33	5	5	1♀	30	—	Fed through a membrane.
16/7/33	4	—	1♂	31	—	—
17/7/33	4	—	—	—	—	—
18/7/33	0	—	1♂, 3♀♀	34	—	Experiment stopped.

TABLE VII.

STARVATION CONTROL
Flies Allowed to Feed Once on Blood and then Starved.

Date.	No. of Flies.	Fed.	Died.	Days. Lived.	Larvae Deposited.	Remarks.
1/10/32	10	8	—	—	—	Fed on blood.
4/10/32	10	—	—	—	1 Abortion...	Flies thin.
5/10/32	8	—	1♂, 1♀	4	—	—
7/10/32	5	—	1♂, 2♀	6	—	Starving.
8/10/32	3	—	2 ♀	7	—	—
9/10/32	3	—	—	—	—	Abdomens wafer-like.
10/10/32	0	—	1♂, 2♀	9	—	—

TABLE VIII.

STARVATION CONTROL.
Flies Allowed to Feed Once on Blood and then Starved.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae Deposited.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
24/10/32	9	—	1 ♀	3	2 Abortions..	—
26/10/32	7	—	1♂, 1 ♀	5	1 Larva......	Starving and weak.
28/10/32	6	—	1♀	7	—	—
29/10/32	4	—	1♂, 1♀	8	—	—
30/10/32	3	—	1♀	9	—	—
31/10/32	2	—	1♀	10	—	—
1/11/32	1	—	1♀	11	—	—
2/11/32	1	—	—	—	—	—
3/11/32	0	—	1♀	13	—	—

One fly died after two days, two after six days, one after twenty days, two after twenty-seven days, one after thirty days, and one after thirty-one days. The remaining four lived thirty-four days. The experiment was then discontinued. Four fully developed larvae were deposited, and five abortions occurred.

From the foregoing it is clear that flies fed through a membrane on blood while not actually thriving, nevertheless can be kept alive for a considerable time, and will even reproduce.

STARVATION CONTROL FLIES.

Two jars of ten flies each were kept as controls. These flies were given one feed of blood and then starved.

Tables VII and VIII show that the longest lived of these starved flies was nine and thirteen days; this is longer than the longest life of any of the flies which had the opportunity to feed on the latex bearing plants. Three abortions occurred and one fully developed larva was deposited. Flies do not readily reproduce when starved, and the one larva was from a female well advanced in pregnancy.

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SUMMARY AND CONCLUSIONS.

(1) This paper gives a brief account of the history of Glossinology. Some habits of Glossina with special reference to *G. pallidipes* have been discussed, together with points of interest in the tsetse's anatomy.

(2) There can be no doubt that the tsetse is strictly haematophagous, and when allowed to feed on blood it will thrive and reproduce. It reacts in a peculiar manner to visual impressions, seeking its food by sight.

(3) The proboscis though a delicate organ, is yet capable of being rapidly thrust deep into the tissues of the insect's animal host.

(4) The complicated process of digestion as studied by Wigglesworth has been briefly discussed, and he has shown that the tsetse's adaptation to a diet of blood has resulted in the great increase of factors affecting blood coagulation.

(5) Further, the tsetse will generally probe against any inert object, provided that object is warm.

(6) Latex, a storage product of certain plants, is contained in lactiferous tubes in the xylem, and the only manner in which the tsetse could feed upon it would be by thrusting its proboscis into a containing vessel.

(7) In the experiments here set forth it is clear that the flies are totally incapable of piercing the plant tissues, and if given opportunity to feed on latex as it occurs in Nature, they soon die and no reproduction takes place.

(8) Further, the latex has an undoubted toxic effect upon those flies which have been artificially induced to partake of it through a membrane.

Section IV.

Mineral Metabolism.

Studies in Mineral Metabolism XXVII.

Modifications of the Methods Used at Onderstepoort for the Determination of (A) Magnesium and Calcium; (B) Potassium, in Grass-Extracts.

By C. R. HOLZAPFEL, M.Sc., Chemist, Onderstepoort.

(A) THE DETERMINATION OF MAGNESIUM AND CALCIUM.

In the course of work on the mineral analysis of grass-extracts, it was observed that the method for the determination of magnesium as described by Malan and Van der Lingen (1931), was not quite satisfactory. Even after changing the washing mixture and washing the final precipitate of magnesium ammonium phosphate with a solution containing ammonia and not sodium hydroxide as described in the original procedure, the method in as far as it was indiscriminately applied to all grass-extracts, still sometimes led to erroneous results. As will be seen later, the chief factor responsible for these errors was shown to be the presence of iron in varying amounts. It should be pointed out here that the concentration of iron in a grass-extract is not necessarily directly proportional to the total amount of iron in the grass, but would appear to be appreciably affected by almost unavoidable fluctuations in ignition temperature and duration, ash composition, etc. It therefore became necessary to modify the magnesium method in such a manner that it would, within reasonable limits, be free of the influence of an unknown and fluctuating iron concentration.

As already indicated, the first step in the present study of the method was directed towards the washing of the final precipitate of magnesium ammonium phosphate. From theoretical considerations, the presence of sodium hydroxide in the washing mixture would tend to decompose the magnesium ammonium phosphate, liberating ammonia, redissolving some of the phosphate and precipitating the magnesium as tri-magnesium phosphate, according to the equation:—

$$3\ MgNH_4PO_4 + 2\ NaOH \rightarrow Na_2NH_4PO_4 + Mg_3\ (PO_4)_2 + 2\ NH_4OH.$$

It can be readily shown that this reaction is practically complete under these conditions of washing. The phosphate equivalent of a magnesium standard, which should read at 29·4 m.m. on the colorimeter against a phosphate standard of 0·2 mgm. P_2O_5 at 30, when all the magnesium was precipitated as magnesium ammonium phos-

phate, was found to give a reading of 42. On the basis of a 100 per cent. transformation to tri-magnesium phosphate according to the above equation, the theoretical reading would be 44. The washing mixture was therefore changed accordingly, 30 c.c. concentrated ammonia (30 per cent.) being added to 1,000 c.c. of the mixture described by Malan and Van der Lingen (1931).

The results obtained with 0·07 mgm. magnesium as read against a 0·2 mgm. phosphate standard at 30 m.m., have been tabulated in Table I. The mixture used for washing the final precipitate was that described above.

TABLE I.

Mg. standard.	Actual colorimetric reading, (average of three readings).	Theoretical reading.
0·07 mgm......................................	29·7	29·4
,, ...	30·0	,,
,, ...	29·9	,,
,, ...	29·4	,,
,, ...	30·0	,,
,, ...	30·1	,,
,, ...	29·5	,,
,, ...	30·2	,,
,, ...	29·7	,,
,, ...	29·7	,,
,, ...	29·3	,,
,, ...	30·1	,,

In the method under review, the magnesium is determined by first precipitating the calcium as oxalate in a solution just alkalinized with ammonia. Without removing this precipitate, the magnesium is then precipitated as magnesium ammonium phosphate by adding excess phosphate and ammonia. The magnesium in this precipitate is determined indirectly by determining its phosphate equivalent colorimetrically. The calcium on the other hand is determined as its phosphate in a second aliquot by directly precipitating the calcium as calcium phosphate from a solution made alkaline with sodium hydroxide.

Since the grass-extract itself contains some phosphate and in addition contains various other metallic ions such as iron, copper, aluminium, manganese, etc., it is clear that a very complex system is being dealt with. With micro-quantities it is possible to choose conditions in such a way that the precipitation of an undesired compound is avoided, either by keeping the product of its ionic concentrations below their solubility product or by utilizing the possibility of complex salts specially characteristic of the micro-concentration range. It follows, however, that any such method must be restricted in its application, since its correctness must be limited to a definite range of concentrations for every individual ion capable of influencing the system.

In dealing with grass-extracts the position is greatly simplified in as much as such metals as aluminium, copper, manganese, etc., are present in such relatively low concentrations that their presence does not in any way appreciably affect the analytical values for calcium and magnesium. In the case of iron the position is different, since grass-extracts frequently contain iron in such quantities, as to lead to serious errors.

Before considering the possible influence of a third element such as iron, it would seem advisable to consider the possible effect of calcium on the magnesium determination. In a neutral solution, containing calcium, magnesium and phosphate with a considerable excess of ammonium oxalate it may be anticipated that only at a relatively high level of phosphate and calcium concentration would some calcium be precipitated as calcium phosphate and thus cause too high magnesium values. In actual experiment it was found that the calcium and initial phosphate had to rise as high as 10 mgm. per aliquot volume before a distinctly measurable error was introduced. This is about five times the amount usually present in grass-extracts.

EFFECT OF IRON ON MAGNESIUM DETERMINATION.

Grass-extracts have been examined with a ferric iron content as high as 24 mgm. Fe per 100 c.c. extract (+10 gm. grass), and possibly higher. Such iron concentrations are sufficiently high to cause at least some of the iron to be precipitated as ferric phosphate under favourable conditions, thereby causing errors in the magnesium values obtained. In the following table the relevant data obtained on standard solutions containing known amounts of magnesium, iron and " initial phosphate " have been compiled, the presence of calcium being maintained merely because it is always present in actual grass extracts.

TABLE II.

No.	Mgm. Mg. present.	Mgm. Ca present.	Mgm. Fe present.	Mgm. (initial) P_2O_5 present.	Product [Fe] × [P_2O_5].	Colorimetric reading.
1	0·10	0·50	0·10	0·40	0·04	20·8
2	0·10	0·50	0·20	0·40	0·08	17·4
3	0·10	1·00	0·10	0·80	0·08	17·0
4	0·10	1·00	0·20	0·40	0·08	16·9
5	0·10	0·50	0·20	0·40	0·08	17·3
6	0·10	0·50	0·30	0·40	0·12	15·4
7	0·10	0·50	0·40	0·40	0·16	13·8
8	0·10	1·00	0·50	0·40	0·20	13·2

According to the quantity of magnesium present, the above colorimetric readings, compared with a 0·2 mgm. P_2O_5 standard at 30 m.m., should all have been in the neighbourhood of 21 m.m. It will be seen that only No. 1 gives a correct reading, all the other readings giving too high values. It will also be noticed that as the product [Fe] × [P_2O_5] increases, the error increases. It may thus be concluded that the presence of iron must either be excluded or

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limited to such amounts that the product [Fe] × ["initial" P_2O_5] is kept well below 0·04 per aliquot volume. The above further suggests that at least some iron is precipitated as ferric phosphate. This conclusion may be confirmed by excluding the magnesium and calcium from the above experiment.

TABLE III.

Mgm. Fe present.	Mgm. (initial) P_2O_5 present.	Product (Fe) × ["initial" P_2O_5.	Colorimetric reading.
0·05	1·0	0·05	Slight colouration.
0·10	1·0	0·10	±80
0·15	1·0	0·15	±60
0·20	1·0	0·20	48
0·25	1·0	0·25	44
0·45	0·8	0·36	25·5
0·55	0·8	0·44	21·6

Obtaining such readings as the above against a 0·2 mgm. P_2O_5 standard at 30 clearly illustrates that at least some iron is precipitated as phosphate since in the above case both magnesium and calcium were absent. The table again suggests that a figure of approximately 0·04 for the product [Fe] × ["initial" P_2O_5] is a maximum value for approximately correct readings. If it is allowed to rise to about 0·4 the error on 0·1 mgm. magnesium would at least be 100 per cent. In solutions containing both iron and phosphate the only safe procedure would thus be either to exclude or remove the iron in some way. It was observed that the addition of oxalate under certain conditions prevented the precipitation of iron, probably in a similar manner as the better known action of citrate on iron in solution. However, the results obtained by exploiting the use of oxalate for preventing the iron from being precipitated were somewhat erratic. The use of citrate proved efficacious as far as keeping the iron in solution, but apparently prevented complete precipitation of the magnesium.

It was therefore decided, first to precipitate the iron as hydroxide (phosphate) by adding ammonia, then precipitating the calcium by adding oxalate, centrifuging off, and determining the magnesium in the supernatant liquid.

The results thus obtained on some standard solutions have been tabulated in Table IV.

TABLE IV.

Mgm. Mg. present.	Mgm. Ca. present.	Mgm. Fe. present.	Mgm. P_2O_5 present (initial).	Product [Fe] × ["initial" P_2O_5.]	Mgm. Mg. recovered.	% Recovery
0·07	0·5	0·3	0·6	0·18	0·067	96
0·07	0·5	0·3	0·6	0·18	0·067	96
0·10	1·0	0·1	0·4	0·04	0·095	95
0·10	0·5	0·3	0·6	0·18	0·098	98
0·10	0·5	0·3	0·6	0·18	0·097	97
0·15	0·5	0·3	0·6	0·18	0·146	97

All these values are satisfactory, since some slight loss was inevitably incurred by the decantation of the supernatant fluid. Such losses, however, are mechanical, and may easily be avoided by taking an aliquot of the supernatant.

To confirm the correctness of this procedure further, and to test its applicability to the analysis of grass-extracts, six such extracts were selected on the basis of their iron content. Using 1 c.c. aliquots, the magnesium was first (A) determined on the calcium oxalate plus ferric hydroxide-phosphate precipitate, then (B) in the supernatant liquid, and finally (C) in a second aliquot on the total precipitate obtained by the original method. These values have been tabulated in Table V.

TABLE V.

Specimen.	A. Mgm. MgO per 100 gm. grass.	B. Mgm. MgO per 100 gm. grass.	C. Mgm. MgO per 100 gm. grass.	Mgm. Fe per 100 c.c. grass-extract.	Mgm. P_2O_5 per 100 c.c. grass-extract.
2	0·08	0·22	0·31	23·5	13·9
13	0·04	0·13	0·18	15·6	9·5
6C (1)	0·00	0·17	0·18	10·3	8·6
44	0·03	0·12	0·15	9·1	9·5
51	0·00	0·20	0·20	7·8	10·3
27	0·00	0·19	0·20	7·7	11·7

In the above table, the values under column B must be considered as correct, those under column A increasing with increase in iron content. When the iron and incidentally the phosphate content is low as in samples 6 C (1), 44, 51 and 27 the difference between columns B and C is either zero or insignificantly small. Where the iron contents are higher, as for example in sample 2, the error is appreciable. The difference between columns C and B, however, more or less exactly equals the figures under column A, whose values were shown to be due to the precipitation of ferric phosphate under the conditions of the original procedure.

After the modification of the method for the determination of magnesium was found to be satisfactory, it seemed possible to determine both calcium and magnesium in the same aliquot of grass-extract, provided the method for calcium determination could be modified accordingly. The method of procedure suggesting itself at this stage was to utilize the precipitate of calcium oxalate for the calcium determination. The precipitate, consisting of calcium oxalate, ferric hydroxide and possibly some ferric phosphate was redissolved in dilute hydrocloric acid, and the calcium in this solution directly determined by precipitation as phosphate according to the original method for calcium. The procedure thus resembles very closely that of Kramer and Tisdall (1921) for the determination of calcium and magnesium in serum, except that the calcium is here determined as phosphate, while according to the method of Kramer and Tisdall the calcium is titrated as oxalate with permanganate. Malan and Van der Lingen (1931) state that the presence of iron,

up to four times the quantity usually present in grass-extracts, has no appreciable effect on the calcium figures obtained. As the following table will show, this statement has been confirmed, correct values being obtained even with iron as high as 0.8 mgm. Fe per c.c. " extract ".

TABLE VI.

Mgm. Mg. present.	Mgm. Ca. present.	Mgm. Fe. present.	Mgm (initial) P_2O_5 present.	Mgm. Mg recovered.	Mgm. Ca recovered.
0·07	0·20	0·10	0·40	0·070	0·200
0·07	0·20	0·10	0·60	0·068	0·198
0·07	0·20	0·20	0·40	0·068	0·203
0·07	0·20	0·20	0·60	0·069	0·203
0·07	0·20	0·30	0·40	0·070	0·204
0·07	0·20	0·30	0·60	0·067	0·203
0·07	0·20	0·50	0·40	0·068	0·204
0·07	0·20	0·60	0·40	0·069	0·200
0·07	0·20	0·80	0·20	0·067	0·194
0·07	0·20	0·80	0·40	0·067	0·192

Incidentally it should be noted that in this procedure the effect of magnesium on calcium determination as stressed by Roe and Kahn (1929) is obviated in as much as magnesium is absent when the calcium is precipitated as phosphate.

As a final test for the applicability of the modified method to grass-extracts, the method has been used for the analysis of grass-extracts to which known amounts of calcium and magnesium have been added. These results have been tabulated in Table VII.

TABLE VII.

Mg present from grass-extract, mgm.	Mgm. Mg added.	Ca present from grass-extract, mgm.	Mgm. Ca added.	Mgm. Mg recovered.	Mgm. Ca recovered.
0·041	0·036	0·074	0·10	0·076	0·174
0·031	0·036	0·101	0·10	0·069	0·198
0·045	0·036	0·148	0·10	0·079	0·232
0·034	0·036	0·114	0·10	0·070	0·214
0·044	0·036	0·158	0·10	0·081	0·246
0·036	0·036	0·072	0·10	0·073	0·172

DETAILED DESCRIPTION OF TECHNIQUE FOR THE DETERMINATION OF MAGNESIUM AND CALCIUM.

1 c.c. of grass-extract is pipetted into a 15 c.c. centrifuge tube. 4 c.c. of distilled water are added and then a minute drop of methyl red. A further drop of strong ammonia (35 per cent.) is sufficient to make the solution just alkaline. After shaking the tube, 1 c.c. of saturated ammonium oxalate solution is added to precipitate the calcium. The tube is then well shaken and left for at least four hours for complete precipitation of calcium. It is then centrifuged

for 10 minutes at about 2,000 r.p.m. The supernatant liquid is then carefully decanted and a 5 c.c. aliquot pipetted into another 15 c.c. centrifuge tube. In this solution the magnesium is determined by precipitating as magnesium ammonium phosphate by adding 1 c.c. of a 1 per cent. mono-potassium phosphate, 2 c.c. of strong ammonia (35 per cent.) and 1 c.c. of a 2 per cent. ammonium chloride. The inside of the tubes are then rubbed with a rubber-tipped glass rod until the precipitate of magnesium ammonium phosphate begins to form. The rest of the procedure is the same as described by Malan and Van der Lingen (1931), employing, however, the new washing mixture described at the beginning of this paper.

The standard to be used in this method, containing appropriate amounts of magnesium and calcium, would be the following:— 0·8 c.c. of a pure solution containing 0·1 mgm. magnesium per c.c. is taken and 1·7 c.c. of a pure solution containing 0·1 mgm. calcium per c.c. is added. The solution is made up to 5 c.c. and proceeded as described above. The magnesium standard obtained in this manner should then theoretically read at 30·7 against a 0·2 mgm. P_2O_5 standard at 30, and the calcium standard at 29·9.

The calculation for the magnesium values of grass would be the following:—

$$\text{gm. Mg. per 100 gm. grass} = \frac{0 \cdot 067 \times 30 \times 6 \times 100 \times 100}{R \times 5 \times v \times g \times 1000}$$

when the 0·067 mgm. magnesium standard is set at 30, R the reading of the unknown, r the aliquot volume of the 100 c.c. grass-extract taken, and g the weight of grass taken for the extract.

The calcium oxalate precipitate in the original tube is then dissolved in 2 to 3 c.c. of N HCl, and the solution made up to 10 c.c. In this solution the calcium is determined as its phosphate, as described by Malan and Van der Lingen (1931). The calcium standard mentioned above is the same as in the original method.

The procedure described above, of determining both calcium and magnesium on the same aliquot of grass-extract is undoubtedly time saving, as it enables one under present conditions, to determine both calcium and magnesium in approximately the same time devoted previously to magnesium only.

SUMMARY.

A modified method for determining calcium and magnesium in grass-extracts is described, and the effect of iron on the original magnesium determination illustrated. Also, a modification of the washing mixture substituting ammonia for sodium hydroxide, for washing the precipitate of magnesium ammonium phosphate was found necessary.

REFERENCES.

KRAMER, B., AND TISDALL, F. F. (1921). A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum. *Jnl. Biol. Chem.*, Vol. 47, p. 475.

MALAN, A. I., AND VAN DER LINGEN, G. W. B. (1931). Studies in Mineral Metabolism, XVI. The Micro-Determination of Some Inorganic Elements in Blood and Vegetation. 17th *Rept.*, *Dir. Vet. Serv.*, Un. of S.A., pp. 443-452.

ROE, J. H., AND KAHN, B. S. (1929). The Colorimetric Determination of Blood Calcium. *Jnl. Biol. Chem.*, Vol. 81, p. 1.

(B) **THE DETERMINATION OF POTASSIUM.**

The alterations adopted in the procedure of this method, as described by Malan and Van der Lingen (1931) resulted from a publication by Roger S. Hubbard (1933). The author advises the standardization of conditions under which the precipitation of potassium is carried out, because potassium and sodium may form a series of double nitrites with cobalt, due to chemical and physical variations.

One c.c. of buffer solution (100 gm. of crystalline sodium acetate made up to 250 c.c. with distilled water) is added to the solution prior to the precipitating agent sodium cobalti nitrite. During the period of precipitation (one hour) the tubes are kept in ice-water, thereby maintaining a constant temperature for precipitation independent of room temperature variations.

Instead of washing the potassium precipitate with distilled water an organic solvent is used (acetone diluted with three parts of water for the first two washings, and pure acetone for the subsequent washing). With this washing fluid the precipitate may be disturbed and easily centrifuged down again, where with pure distilled water some of it would invariably remain on the water surface in spite of centrifuging. These alterations have been applied to the potassium determination with advantage.

REFERENCES.

HUBBARD, R. S. (1933). Note on the Precipitation of Small Amounts of Potassium as Potassium Sodium Cobalti-Nitrite. *Jnl. Biol. Chem.,* Vol. 100, pp. 557-559.

MALAN, A. I., AND VAN DER LINGEN, G. W. B. (1931). Studies in Mineral Metabolism, XVI. The Micro-Determination of some Inorganic Elements in Blood and Vegetation. *17th Rept., Dir. Vet. Serv.,* Un. of S.A., pp. 446-447.

APPENDIX.

Since the above was written, it was found necessary to determine the calcium and magnesium contents of not only grasses but of other feeding stuffs like maize, beans, etc., in which the limits of variation of these elements were wider than those given. Under such conditions the method could not be applied wholly as given and slight modifications had to be introduced.

First of all, in the combined determination of calcium and magnesium, the calcium concentration in the aliquot should not exceed 0·25 mgm. (see Table VII) but should rather be kept between 0·1 mgm. and 0·2 mgm. As described above, the calcium oxalate precipitate is dissolved in HCl and then the calcium is again precipitated as calcium phosphate in an alkaline medium. Under these conditions, with a higher calcium concentration than that given, viz., 0·1 mgm. to 0·2 mgm. per aliquot, and an equivalent concentration of oxalate present, some calcium oxalate may again be precipitated in the alkaline medium when the calcium is precipitated as phosphate.

In other cases, when both magnesium and phosphate are exceptionally high, some magnesium may be precipitated as $Mg NH_4 PO_4$ when precipitating the calcium oxalate. This difficulty may be overcome by taking a very small aliquot of the extract, or by precipitating the calcium oxalate at a pH between 3 and 4 when the magnesium remains in solution. Under these conditions, however, the iron concentration in the aliquot should be very low or nil, else the iron would eventually be precipitated as iron phosphate along with the $Mg NH_4 PO_4$.

In the event of it being impossible to take an aliquot to suit the determination of both calcium and magnesium, the determinations should be carried out on separate aliquots, the calcium being determined directly as calcium phosphate as described by Malan and van der Lingen, and the magnesium as $Mg NH_4 PO_4$ according to the procedure described in the article given above.

Studies in Mineral Metabolism XXVIII.

Methods for the Micro-Determination of Iodine in Biological Material.

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ONE of the chief difficulties in iodine investigations in the past has been the lack of a method of analysis, sufficiently delicate, to determine accurately the very small amounts of iodine present in some biological substances. The literature relating to the methods proposed in the past has been reviewed so comprehensively by Scharrer (1928) that the older methods will not be discussed here. The modern methods can be arranged into three groups depending on the primary treatment of the material to be analysed. The material is decomposed so that iodine can be extracted in the form of a water-soluble compound. The methods can be classified as follows:—

(a) *Dry Oxidation in a Closed System.*—According to McClendon (1927), Schwaibold and Harder (1931), etc., the material is heated in a closed system in a current of oxygen, and the combustion products passed through an electrical precipitator or over a heated catalyst and finally through an alkaline solution which absorbs the iodine.

(b) *Nickel Dish Method.*—The method adopted by Van Fellenberg (1926) and modified by Scheffer (1930) and others, lays down that the material shall be mixed with an alkali in an open—usually nickel—dish and heated carefully until all the carbonaceous matter has been charred or totally oxidized. The iodine in the form of an alkali iodide is then extracted with ± 95 per cent. alcohol, or with water and alcohol.

(c) *Wet or Acid Oxidation Method.*—Pfeiffer (1930) proposed the use of this method. The material is treated with perhydrol and concentrated sulphuric acid in a closed system and the iodine liberated is absorbed in an alkaline solution.

The numerous methods cited in the literature bear testimony to the fact that some essential detail is lacking in the procedure, and lately, workers have concentrated on perfecting the existing methods rather than proposing new ones, so that iodine can now be determined with a fair degree of accuracy by the experienced worker.

Although the greatest care should be taken in the determination of iodine when it has been converted into a soluble form free from organic matter, all possible precautions should be adopted in the primary treatment of the material to avoid loss of iodine; and, until such time as the carbonization in open crucibles has been perfected, the dry oxidation in a closed system should yield the best results,

although, unfortunately, it is very lengthy and therefore hardly suitable for routine analyses. A criticism against the use of the acid oxidation method in the analysis of plant material is the possible error which may be introduced by impurities, which, in the form of iodine, may be present in the large amount of reagents used. Consequently, it was decided to test the accuracy of the nickel dish method, the most rapid and hence the most adaptable for routine analysis, by comparing its efficiency with that of the dry oxidation method as a standard. The procedure adopted in the technique is as follows:—

Closed System Method.

About 20 to 30 grams of dry material (grass, vegetables, etc.), are packed into a clear silica tube 28 inches long and one inch internal diameter. The tube is fitted with a rubber stopper at each end. The primary oxygen is led in at the one end and the secondary supply at the other end through a silica tube which passes through the catalyst consisting of silky fibrous asbestos. The material for analysis is kept in position by a plug made from Whatman No. 40 filter paper. The ashing is carried out as follows: The primary oxygen is turned on until the air in the tube and wash bottles is displaced. The catalyst is then heated by means of gas burners and when the temperature reaches $\pm 700°$ C. the secondary oxygen is also turned on. The organic material furthest away from the catalyst is heated slowly until combustion starts. No more external heat is applied to the combustible material for some time and a combustion zone travels slowly along the length of the tube. Some carbon is deposited on the inside of the tube and this is finally burnt away by applying external heat.

The combustion products travel along the tube until they come into contact and mix with the secondary oxygen, and then pass through the heated catalyst where complete combustion takes place. In this way the tarry vapours are all destroyed and the oxidation products, which are now colourless, plus the excess secondary oxygen pass through wash bottles containing solutions of potassium carbonate. By including two of these absorption flasks in the circuit all the iodine contained in the combustion products is absorbed.

When all the carbonaceous matter is oxidized, the residue is cooled in a current of oxygen. The tube is then washed with distilled water and the combined washings, to which is added 0.5 c.c. saturated potassium carbonate, heated on the steam bath for some time and filtered. The contents of the wash bottles are poured into a platinum dish, the bottles washed with distilled water and these washings, together with the wash water from the silica tube, are added to the contents of the platinum dish. The solution is evaporated to dryness, the contents of the dish glowed over an open flame to remove the last traces of organic material, dissolved in a minimum quantity of water, and filtered. The dish is washed with small quantities of water which, after being filtered, are added to the main filtrate. The alkaline filtrate is neutralized with hydrochloric acid and a drop of excess acid added. After the addition of a few grains of pumice stone and about 1 c.c. freshly-prepared chlorine water the excess chlorine is boiled off. This is accomplished

after 10 minutes' brisk boiling. When the solution is cold a very small crystal of potassium iodide (about 0.001 gm.), and about 10 drops of a ·25 per cent. starch solution are added and the iodine liberated is titrated against thiosulphate using a micro-pipette.

Chlorine water is prepared a few minutes before it is required by dropping concentrated hydrochloric acid on to potassium permanganate and passing the chlorine through distilled water contained in a conical flask fitted with an outlet tube. When the chlorine water is required, this tube is pushed down so that it reaches into the water. The positive pressure in the apparatus forces the chlorine water out drop by drop.

Blank determinations were carried out on the reagents employed. The same procedure as in the actual determination was adopted and also the same quantity of distilled water and reagents used. Iodine was not detected in any of these blanks. This showed definitely that after 10 minutes boiling all the chlorine had been driven off.

The asbestos used as catalyst is purified by heating it with concentrated sulphuric acid for twelve hours and washing it thoroughly, first with tap water and finally with distilled water. The asbestos is then dried and heated at ±600° C. to remove the last traces of the acid.

The closed system method is taken as a standard, i.e. one which gives us nearly absolute results as possible. Loss of iodine due to escape during the combustion process is eliminated, and after oxidation all the iodine is present either in the silica tube or else in the absorption flasks. In either case the final solution, i.e. washings from silica tube plus absorption liquid, contains all the iodine. The method gives excellent results on duplicate determinations and on recovering added iodine compounds as described in Tables I and II.

It was therefore decided to test out a simpler method suitable for routine analysis and to standardize it against the silica tube method. The manipulation of the apparatus employed in the acid oxidation (Pfeiffer, 1930) is too complicated and besides being unsuitable for bulky plant materials low in iodine, it offers no advantage over the dry oxidation in a closed system. The only alternative is the partial ashing of organic material in the presence of an alkali in open crucibles or dishes.

NICKEL DISH METHOD.

The method adopted is based on that described by Scheffer (1930) with slight modifications. A sample of finely ground dry plant material—about 20 to 60 gm., depending on the anticipated iodine content of the substance analysed—is weighed into a nickel dish and completely covered with water. To this 5 to 15 c.c. of a saturated potassium carbonate solution are added, and the contents stirred with a glass rod so that the alkali is mixed intimately with the individual particles of the organic material. The excess water is evaporated on the steam bath and the nickel dish placed in a large electric furnase the temperature of which can be regulated by means of a suitable resistance in the circuit. The current is switched on and the temperature raised to ±150° C. The resistance is then increased so that

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the furnace is heated very gradually. On account of the swelling of starchy materials the procedure adopted in the case of grass and fibrous materials is slightly different to that for intumescent materials.

GRASS AND FIBROUS MATERIALS.

At approximately 250° to 270° C. the contents of the dish begin to glow and an ignition zone travels through the entire mass. The current is switched off as soon as spontaneous combustion takes place, and it is found that under these conditions the temperature of the furnace can be regulated not to exceed 290° C., although that of the glowing mass in the nickel dish may be slightly higher. This is important because the efficiency of the method depends largely on the temperature employed and at no stage during the carbonization should this exceed 300° C.

INTUMESCENT MATERIALS.

These substances swell to an enormous extent when heated above 200° C. To overcome this the dish is cooled from time to time and the contents broken up with a nickel spatula. This process is continued till intumescence ceases. The dish is then heated further to 290° C. and kept at this temperature until the material is totally charred.

When all the organic matter—fibrous and starchy—is carbonized, the dish is allowed to cool, the contents finely powdered and transferred to a conical flask. The dish is washed with small quantities of water and the washings added to the contents of the flask. The flask is heated on the steam bath, 50 c.c. absolute alcohol added, the flask shaken with a gyratory motion and the alcohol extract filtered. The residue is extracted with three successive portions of alcohol and finally it is transferred to the filter and washed with alcohol.

The alcohol extract plus 2 c.c. saturated potassium carbonate solution is slowly evaporated to dryness in a platinum dish without allowing the contents to boil. The dish is gently glowed over an open flame and the residue moistened with water and extracted with successive portions of alcohol until the alkali separates out. The residue is again moistened with water, extracted with alcohol as before and the filtered alcohol extract added to the main extract.

It is of the utmost importance in the extraction of potassium iodide to dissolve all the soluble material in a minimum quantity of water so that a fairly saturated solution is obtained, and then only to add the alcohol. In this way the alcohol is brought into direct contact with the iodide which cannot happen when a mixture of water and alcohol is used for extraction. In the latter case the iodine may be so occluded by the rest of the material present that extraction can only be complete when the material is ground to an impalpable powder. Scheffer (1930) illustrated this fact very clearly. Also Hercus and Aitken (1933) in studying the partition of potassium iodide at low concentrations between alcohol and potassium carbonate solution found that if the potassium carbonate was saturated or nearly

so and excess absolute alcohol added to dehydrate the paste, the partition coefficient was of such an order that under the conditions described in this paper for the extraction all the iodide should be present in the alcohol extract.

The filtered alcohol extract plus 0·5 c.c. potassium carbonate solution is transferred to a platinum dish, slowly evaporated to dryness and gently glowed. The residue is dissolved in a few c.c. water, the solution filtered, the dish washed three times with a few c.c. water, and the washings added to the main filtrate. The combined filtrate is neutralized with hydrochloric acid and a drop excess acid added. The rest of the procedure is similar to that described for the closed system method.

STANDARDIZING AND COMPARING THE EFFICIENCY OF THE METHODS.

The efficiency of the silica tube method was determined by adding 1 c.c. potassium iodate solution of known strength to filter paper (free from iodine) and also to grass and analysing the materials according to the closed system method. In some cases the iodine present in the two absorption flasks and in the washings of the silica tube was determined separately, while in others the various iodine containing fractions were all combined.

TABLE I.

The Efficiency of the Closed System Method.

Substance Analysed.	Weight of Material Analysed. Gram.	Iodine Present in Material.†γ.	Iodine Added in the Form of KIO₃. †γ.	Total Iodine Present.†γ	Iodine in First Absorption Flask.†γ	Iodine in Second Absorption Flask. †γ	Iodine in Washings from Silica Tube.†γ	Total Iodine Recovered. †γ	Percentage Recovery.
Two filter papers...............	—	—	80	80	73·8	1·0	4·5	79·3	99
Two filter papers...............	—	—	80	80	74·2	0·9	3·6	78·7	98
Two filter papers...............	—	—	80	80	75·6	0·7	3·6	79·9	100
Mature Grass (18)...............	9·3	*9·7	80	89·7		80		80	89
Green Grass (28)...............	11·0	*7·3	80	87·3		78·6		78·6	90
Mature Grass (14)...............	12·8	*8·0	80	88·0		79·3		79·3	90
Mature Grass (14)...............	12·6	*7·9	80	78·9		78·7		78·7	90

These results need some explanation because the total iodine recovered is just equal to or less than that added to the grass. At first sight it may thus appear that none of the iodine originally present in the grass is recovered. This, however, is certainly not true and the explanation is offered in Table III. Here the same amount of iodine is added to samples of grass and where the iodine present in the original sample exceeds 12 γ the iodine recovered is always more than that added, even although the percentage recovery is less than that recorded in Table I. It is clear, therefore, that the iodine recovered is made up of that present in the material analysed as well as that added in the form of potassium iodate.

* The values used here are calculated from the results obtained for the closed system method *vide* Table II.

† The sign γ is used to denote a microgram or the millionth part of a gram.

The following table of results compares the efficiency of the nickel dish method with that of the dry combustion method:—

TABLE II.

Substance Analysed.	Dry Combustion Method.					Nickel Dish Method.	
	Iodine in First Absorption Flask. $\gamma\%$	Iodine in Second Absorption Flask. $\gamma\%$	Iodine in Washings from silica tube. $\gamma\%$	Total Iodine. $\gamma\%$	Average. $\gamma\%$	Total Iodine in Material. $\gamma\%$	Average. $\gamma\%$
Mature Grass (18)......	94·8	2·8	7·0	106·6		102	102
Mature Grass (18)......	84·1	4·1	15·7	103·9	104·6		
Mature Grass (18)......	88·8	4·9	11·6	105·3			
Mature Grass (14)......	54·3	3·8	4·7	62·8	62·8	60	60
Green Grass (28).......	43·3	3·9	17·6	65·8		62·3	62·3
Green Grass (28).......	40·8	3·9	18·5	63·2	65·6		
Green Grass (28).......	34·1	4·0	29·6	67·7			
Mature Grass (20)......		161·			161	152, 164	158
Mature Grass (40B).....		111·5			111·5	114·1	112
						110·7	
						110·8	
						112·4	
Potatoes (S. 990).......		79·0			79·0	83·0	82·4
						79·5	
						82·5	
						84·6	
Potatoes (S. 991).......		92·2			92·2	110·8	112
						113·2	
Wheat (S. 9891)........		10·1			10·1	10·5	10·2
						10·0	
Wheat (S. 989 II)......		16·3			16·3	14·8	14·5
						14·7	
						14·0	

The results obtained by the nickel dish method agree fairly well with those of the standard method and lie within the limits of experimental error. The efficiency of the nickel dish method was also determined in a more direct manner. One cubic centimeter potassium iodate solution of known strength was again added to the organic material and the analysis carried out as already described. The results given in Table III show that the percentage recovery is not much lower than that obtained with the closed system, *vide* Table I.

TABLE III.

Nickel Dish Method.

Material.	Weight: gram.	Iodine Present. γ	Iodine Added. γ	Total Iodine. γ	Iodine Re-covered. γ	% Re-covery.
Mature Grass (14).............	12·1	7·6	80	87·6	73·7	84
Mature Grass (14).............	10·8	6·8	80	86·8	74·4	86
Mature Grass (20).............	13·8	21·8	80	101·8	84·5	83
Mature Grass (20).............	15·0	23·7	80	103·7	83·6	81
Mature Grass (40в)...........	14·2	15·9	80	95·9	87·0	90
Mature Grass (40в)...........	17·1	19·2	80	99·2	94·1	95
Mature Grass (40в)...........	11·3	12·7	80	92·7	82·8	89
Mature Grass (40в)...........	10·3	11·5	80	91·5	80·3	88

Emphasis has been laid on the fact that the maximum temperature employed during the carbonization stage in the nickel dish method should not exceed 300° C. Widmann (1932) has shown that when a temperature range of 300° to 350° C. is employed, the values for blood average only 36 per cent. of those obtained with an absolute method, while in the case of a range 200° to 250° C. the average figure is 88 per cent. This figure agrees very well with the average percentage recovery of Table III, which is 87 per cent.

It was found that, if the temperature is allowed to exceed 300° C. for any length of time, as much as 60 per cent. of the iodine present is lost. This is borne out by the results tabulated below:—

TABLE IV.

Material.	Silica Tube Method. $\gamma\%$	Nickel Dish Method.	
		250° to 300°C. $\gamma\%$	±400° C. $\gamma\%$
Wheat I.................................	10·1	10·2	7·5, 8·2
Wheat II................................	16·3	14·5	13·2
Mature Grass (20)......................	161·0	158·0	87·3, 97·5
Mature Grass (14)......................	62·8	60·0	51·0
Mature Grass (28)......................	65·6	62·3	48·1

Tables II and III illustrate the efficiency of the nickel dish method as long as the specified temperature range is adopted and the alcohol extraction carried to completion. For this reason as well as the suitability of the method for routine work, this method was adopted throughout the present investigation and the same procedure followed with slight modifications to suit the requirements of the different types of materials analysed.

Summary.

Micro-analytical methods for the determination of iodine in biological substances are discussed and the efficiency of the nickel dish method compared with that of the dry oxidation in a closed system. It is shown that under the conditions specified the error in duplicate analyses does not exceed 20 per cent., while over 80 per cent. of the total iodine present, i.e. iodine in the orginal material plus the amount added, can be recovered.

LITERATURE.

HERCUS, C. E., AND AITKEN, H. A. A. (1933). *Jour. of Hyg.*, Vol. 33, pp. 55-79.

McCLENDON, J. F. (1927). The Distribution of Iodine with Special Reference to Goitre. *Phys. Reviews*, Vol. 7, pp. 189-252.

PFEIFFER, G. (1930). Die Zusatzverbrennung zur sauren Jodbestimmungsmethode in organischen Produkten. *Bioch. Zeit.*, 228, pp. 146-153.

SCHARRER, K. (1928). Chemie und Biochemie des Jods.

SCHEFFER, L. (1930). Mikro-jod-bestimmung in organischen Substanzen. *Bioch. Zeit.*, No. 228, pp. 426-436.

SCHWAIBOLD, J., AND HARDER, B. (1931). Die Bestimmung des Jods in biochemischen Materialien. *Bioch. Zeit.*, No. 240, pp. 441-453.

VON FELLENBERG, TH. (1926). Das Vorkommen, der krieslauf und der Stoffwechsel des Jods. *Ergebn. der Phys.*, No. 25, pp. 176-363.

WIDMANN, E. (1932). Zur Methodik der Blutjodbestimmung. *Bioch. Zeit.*, No. 254, pp. 223-228.

Studies in Mineral Metabolism XXIX.

The Iodine Content of Foodstuffs in Relation to the Occurrence of Endemic Goitre in the Langkloof Valley.

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THE literature relating to the problem of iodine metabolism with and without regard to deficiency diseases has been reviewed so comprehensively in recent years that a general discussion will not be attempted in this paper. Only work having a direct bearing will be considered. Von Fellenberg (1926), McClendon (1927), Scharrer (1928), and Orr and Leitch (1929) have all given full accounts of the existing literature, while Hercus and co-workers (1925, 1927, 1931) published valuable information regarding the problem of endemic goitre in New Zealand.

In Switzerland Von Fellenberg found an inverse relationship between the iodine content of foodstuffs and the incidence of goitre, and this has been confirmed by McClendon in America, and Hercus and co-workers in New Zealand. Orr and co-workers, however, were unable to trace any definite correlation between the level of iodine in drinking-water and foods and the incidence of goitre in the British Isles, but judging by the rôle of iodine in the treatment and prevention of the disease they surmise that deficiency of iodine may have a causal relationship to overgrowth of the thyroid. Scheffer (1932), on the other hand, believes that while deficiency of iodine may be a contributing factor for the development of goitre, there are other and perhaps more important constitutional factors.

OUTLINE OF THE INVESTIGATION.

Following a report in 1929 by the medical officer of health for the Union, "Endemic Goitre in the Komgha Valley, Uniondale District" the Senior Chemist in charge of the Government Chemical Laboratories, Pretoria, obtained samples of water, vegetation and foodstuffs from this area for analysis. Unfortunately this program could not be carried out and nothing further was done until the present investigation was started.

In his report the medical officer mentions the high incidence of goitre in some of the valleys on either side of Langkloof, especially in the neighbourhood of Joubertina. The inhabitants of some of these valleys were medically examined and the following figures taken from the report represent the positive goitres encountered:—

Hoeree Valley 79%
Children at Kleinrivier school 93%
Children at Krakeel school 69%

At Joubertina Secondary School no obvious goitres were encountered except in the case of those pupils whose homes are in the valleys where goitre occurs.

It was decided to visit these valleys personally to collect samples of food in order to safeguard against iodine contamination in transit. The tour was undertaken in September, 1932, so that samples of leafy vegetables and grass could be obtained in an immature stage.

Two of the goiterous valleys—Hoeree and Kleinrivier—were visited. They run more or less parallel to each other and at right angles to Langkloof. They lie between longitude east 24° and 24° 15′ and latitude south 33° 40′ and 33° 50′. The Langkloof Valley lies along the course of the Kouga River between the Kouga and Langkloof Mountains near the south coast of the Cape Province, and stretches from George District through Uniondale District to Humansdorp District for approximately 100 miles. Both the Hoeree and Kleinrivier streams originate in the Kouga Mountains and are tributaries of the Kouga River. The geological formation is Table Mountain sandstone. Entrance to the valleys is along the river beds and in times of flood these roads are impassable and communication with the outside world is cut off.

The valleys are very narrow, the sides precipitous and about 1,500 feet high. Furthermore, they extend north-south so that some of the dwelling-houses only have a few hours sunshine daily. The inhabitants are extremely poor and are dependant on the produce grown locally for their food, except meat, which is obtained from the Joubertina butchery when the necessary funds are available. Meat, however, is considered a delicacy and is available only about once or twice a month. On the whole very little green vegetables are eaten.

The daily diet is made up as follows:—

Breakfast.—Black coffee usually without sugar with dry bread and wheat meal or mealie meal porridge, very often without the addition of milk or sugar.

Dinner.—Bean or potato soup, samp or potatoes depending on the variety of soup, dry bread and coffee. The soup is usually prepared without the addition of meat or meat extract.

Supper.—The food left over from the previous meal is consumed in the evening.

This menu clearly demonstrates that a very large precentage of the diet consists of starchy food and the main sources of proteins are beans, wheat, meat and eggs, the latter two only eaten occasionally. Very little, if any, leafy vegetables are eaten and the children prefer bread and dripping to greens. Oranges and mandarines are grown to some extent and these are relished while the season lasts. It is interesting to note that this diet corresponds closely with that described by Bodnár and Straub (1930) for the inhabitants of Bodahegyközség in Hungary, where the incidence of goitre amongst children at school is 82 per cent.

Samples of water, food, pasture and soil were collected from the two valleys and transported to Onderstepoort where the samples of food and pasture were dried in the shade and prepared for analysis.

During May this year Mr. Du Toit, the Extension Officer at Humansdorp kindly collected some more samples of foodstuffs from Hoeree and Kleinrivier and also from Twee River, a non-goiterous valley in the neighbourhood of the other two valleys. These samples represent the crop for the 1932-1933 season. For comparative purposes samples of foodstuffs were obtained from different parts of South Africa where endemic goitre is unknown. The food was produced in the areas stipulated and should not be looked upon as market samples.

The results are given in Tables I to V, together with some figures taken from the literature. They are summarized in Table VI.

TABLE I.

Iodine Content of Eggs.

Origin of Sample.	Nature of Area.	Iodine expressed as γ per 100 gm. fresh weight.
Joubertina { Hoeree........	G....................	5·2, 4·8, 4·6.
Kleinrivier......	G....................	4·1, 4·1.
Tweerivier......	NG....................	10·5, 9·7.
Graaff-Reinet................	NG....................	31, 31·5, 51.
Montagu....................	NG....................	10·2, 5·3, 12·7.
Louis Trichardt.............	NG....................	8·6, 11·0, 13·0, 18·2.
Pretoria, Onderstepoort......	NG....................	5·9, 7·1.
Pretoria....................	NG....................	3·9, 5·5, 6·0, 7·0, 8·0.
Pretoria, Pyramid...........	NG....................	6·1, 7·2, 7·5.
	From Literature.	*Investigator.*
Switzerland.................	NG....................	21·5 v. Fellenberg (1923 & 1924).
Switzerland.................	G....................	8·0, 14·7 v. Fellenberg (1923 and 1924).
Europe.....................	Market samples......	1·2, 2·2, 2·7 v. Fellenberg (1923 and 1924).
New Zealand................	NG. Average of 18..	13·7 Hercus and Roberts (1927).
New Zealand................	G. Average of 20....	5·6 Hercus and Roberts (1927).
Scotland....................	N.G. Average of 41..	10·2 Orr (1930).
Scotland....................	G. Average of 15....	8·6 Orr (1930).
England....................	Low incidence Average of 56	6·9 Orr (1930).
England....................	High incidence Average of 52	8·5 Orr (1930).

G. represents Goitre area and N.G. areas where endemic goitre is unknown.

TABLE II.

Iodine Content of Potatoes.

Origin of Sample.		Nature of Area.	Water Content %	Iodine expressed as γ per 100 gm.	
				Fresh Weight.	Dry Weight.
Jouber-tina	Hoeree (1929)....	NG	—	—	113, 111.
	Hoeree (1929)....	G	—	—	104, 107, 113, 113.
	Hoeree (1932)....	G	79	3·3, 4·2	15·7, 20·0.
	Hoeree (1933)....	G.	77·7	28·3	127·0.
	Kleinrivier (1929)	G.	—	—	79, 82·5, 85.
	Kleinrivier (1932)	G.	75	4·9, 5·8	19·6, 23·2.
	Kleinrivier (1933)	G.	78·9	34·3	163·0.
	Twee River (1933)	NG.	78·2	27·8	127·3.
Graaff Reinet..............		,,	77	17·3	75·2.
Montagu...................		,,	73·8	8·7	33·2.
Pietersburg...............		,,	78	21·6	98·2.
Machadodorp..............		,,	75·3	5·5	22·3.
Pretoria	Pretoria North...	,,	80·5	2·9	14·9
	Silverton........	,,	78·2	3·0, 3·1	13·8, 14·2.
	Daspoort No. 1..	,,	80·4	20·5, 16·4	105, 84.
	Eerste Fabrieke..	,,	78·5	19·6	91·2.
	Pyramid.........	,,	79·6	15	73·5.
	Eloffsdal........	,,	78·4	24·5	113.
	Daspoort No. 2..	,,	77·8	2·9	13·1.
Pretoria District...........		,,	77·0	21·5	93·5.
		From Literature.			*Investigator.*
Hungary...................		G.	—	8·35	Scheffer (1932).
Hungary...................		NG.	—	1·5	Bodnár and Straub (1930).
Hungary...................		G.	—	0·5	Bodnár and Straub (1930).
Austria...................		Market sample, Vienna	—	1·0–1·8	Mayhofer, etc. (1932).
New Zealand..............		Market sample	—	1·0, 2·2	Hercus and Roberts (1927).
Germany..................		Market sample	80·9	5·0	Scharrer and Schwaibold (1928).
Scotland..................		NG.	Average of 5 samples	9·6	Orr.
England..................		Low incidence	Average of 10 samples	10·0	Orr.
England..................		High incidence	Average of 7 samples	10·3	Orr.

TABLE III.
Iodine Content of Wheat.

Origin of Wheat.		Nature of Area.	Water Content %	Iodine expressed as γ per 100 gm.	
				Fresh Weight.	Dry Weight.
Jou-bertina	Hoeree (1929)....	G.	—	—	10·1, 10·5, 11·0.
	Hoeree (1932)....	G.	11·7	8·5, 9·2, 9·6	9·6, 10·4, 10·9.
	Hoeree (1933)....	G.	11·9	4·3	4·9.
	Kleinrivier (1932)	G.	10·7	15, 15	16·8, 16·8.
	Kleinrivier (1933)	G.	12·0	3·8	4·2.
	Tweerivier (1933)	NG.	12·4	3·8	4·3.
Graaff Reinet.............		NG.	9·5	3·3	3·6
Montagu..................		NG.	10·9	5·0	5·6
Lydenburg................		NG.	10·6	10·2	11·4
Ceres....................		NG.	10·3	3·9	4·3
Ficksburg................		NG.	9·8	1·8	2·0
Pietersburg..............		NG.	11·3	2·4	2·7
Pretoria.................		NG.	8·4	3·1	3·4
		From Literature.			*Investigator.*
U.S.A....................		Market sample	—	4·8, 6·4	v. Fellenberg (1924 2.)
Canada..................		,,	—	0·3, 2·6, 5·6	v. Fellenberg (1923)
South America...........		,,	—	0·2, 2·8	,, ,,
Australia................		,,	—	1·9, 4·4	,, ,,
Roumania................		,,	—	0·2, 2·8	,, ,,
Switzerland..............		,,	—	2·6, 2·7, 4·0	,, ,,
Wheat Flour Germany......		NG.	—	14·2, 9·5	Bleyer (1926).
Wheat Flour Germany......		NG.	—	9·3	Bleyer (1926).
Wheat Flour Hungary.......		G.	—	12·0	Scheffer (1932).

TABLE IV.
Iodine Content of Mealies.

Origin of sample.		Nature of Area.	Water Content. %	Iodine expressed as γ per 100 gm. on.	
				Fresh Weight.	Dry Weight.
Jou-bertina	Kleinrivier (1932)	G.	10·3	5·2, 5·2, 5·4	5·8, 5·8, 6·0
	Kleinrivier (1933)	G.	10·1	3·8	4·2
	Hoeree (1933)....	G.	11·1	4·7	5·3
	Tweerivier (1933).	NG.	12·0	3·8	4·3
Graaff Reinet.............		NG.	11·3	4·0	4·5
Montagu..................		NG.	10·5	3·2	3·6
Pretoria District		NG.	10 8	11 3	12·7
Pretoria District..........		NG.	8·7	2·4	2·6
Pretoria District..........		NG.	8·0	1·8	2·0
Heilbron.................		NG.	8·5	3·9	4·3
Pretoria.................		Market sample	8·1	3·8	4·1
Pretoria.................		,,	10·1	2·4	2·7
Pretoria.................		,,	9·0	1·9	2·1
Pretoria.................		,,	10·5	3·3	3·7
		From Literature.			*Investigator.*
Italy....................		Market sample	—	1·2	v. Fellenberg (1924. 2).
Germany.................		,,	12·9	9·0	Scharrer and Schwaibold (1928).

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TABLE V.

Iodine Content of Dry Beans.

Origin of Sample.	Nature of Area.	Water Content %	Iodine expressed as γ per 100 gm.	
			Fresh Weight.	Dry Weight.
Joubertina — Hoeree (1932)....	G.	11·3	5·0, 5·3, 5·7	5·6, 6·0, 6·4
Hoeree (1933)....	G.	11·8	4·6	5·2
Kleinrivier (1932)	G.	11·8	3·4, 4·0, 4·4	3·9, 4·5, 5·0
Kleinrivier (1933)	G.	11·1	3·2	3·6
Tweerivier (1933)	NG.	10·9	4·7	5·3
Graaff Reinet............	,,	9·3	7·5	8·3
Montagu.................	,,	11·2	6·7	7·5
Ceres....................	,,	9·7	7·1	7·9
Western Cape Province.....	,,	9·5	6·7	7·4
Hylands Natal............	,,	8·5	5·8	6·3
Pretoria.................	,,	9·5	6·1	6·7
Nylstroom...............	,,	8·5	1·95	2·1
" Soya beans " Hylands Natal	,,	5·9	3·6	3·8
	From Literature.			*Investigator.*
Hungary..................	G.	—	26·4	Scheffer (1932).
U.S.A....................	G.	—	2·9	McClendon & Hath (1924).
Hungary..................	NG.	—	0·8	Bodnár & Straub (1930).
Switzerland..............	G.	—	2·5	v. Fellenberg (1926).
Austria..................	Market	—	3·9	Mayrhofer, etc. (1932).
Germany.................	Market	19·5	9·0	Scharrer & Schwaibold (1928).
" Soya beans," Germany....	Market	11·2	17·0	Scharrer & Schwaibold (1928).

TABLE VI.—SUMMARY OF RESULTS.

Iodine expressed as γ per 100 gm. fresh weight of eggs and per 100 gm. dry weight of other foodstuffs.

Foodstuff.	Goitre free areas.		Goitre areas.	
	Range.	Average.	Range.	Average.
Eggs.................	3·9–51·0	13·2	4·1–5·2	4·5
Potatoes.............	13·1–127·5	66·4	15·7–162	90·3
Wheat...............	2·0–11·4	4·7	4·2–16·8	9·3
Mealies..............	2·0–12·7	4·9	4·2–6·0	5·2
Dry Beans...........	2·1–8·3	6·2	3·6–6·4	5·0

One of the main essentials in a study of the relationship between the iodine content of foodstuffs and the incidence of goitre is the existence of isolated endemic goitre areas where the inhabitants are dependent on the produce grown locally. These conditions usually only prevail in the case of small islands and valleys not easily accessible. Small inhabited islands, however, are usually free from goitre and the modern methods of transport leave few valleys inaccessible. Consequently a large amount of the food consumed in goitre areas can be procured from somewhere else and, although locally grown samples are collected, they may not represent the food of the inhabitants.

Geographically the Hoeree and Kleinrivier Valleys are practically inaccessible, and as the inhabitants are very poor, they are forced to rely on the produce grown locally for their existence. The high incidence of goitre prevailing in these valleys naturally makes them ideal goitre areas from which to collect samples of food for iodine analysis. Consequently, it was anticipated that, if goitre is due primarily to deficiency of iodine, it would be possible to trace some relationship between the iodine content of food and the occurrence of goitre.

Tables I to V show that no such relation exists. The only foodstuffs of which the iodine content is lower for the goitre areas than that for the other areas are eggs and dry beans, but this difference is more than compensated for by the high iodine content of potatoes and wheat, which form the staple diet of the inhabitants. In normal areas also, such wide variations are encountered in the iodine content of potatoes, eggs, and beans, that the occurrence of goitre cannot be ascribed primarily to the lower iodine content of eggs and beans obtained from the valleys.

The iodine content of plants is dependent on various factors, e.g. the amount and state of the iodine present in the soil, climatic conditions and perhaps the variety of a particular type of plant. It is not known to what extent these different factors contribute to the fixation of iodine by the plant. Possibly the iodine content is also governed by the stage of growth in a manner similar to that obtained in the case of grass which is described in the following paper. Some work has already been done in this connection, but the results do not warrant any definite conclusion at this stage.

It is interesting to note that, although very wide variations are met with in the case of potatoes and wheat, the iodine present in dry beans and mealies remains fairly constant. Only one sample of mealies was found to be high in iodine and this sample was collected before maturity was reached.

SUMMARY.

Iodine was determined in foodstuffs from different parts of South Africa including those from endemic goitre areas in the Uniondale district, and such widely differing results were obtained that no fixed relationship could be established between the iodine content of foodstuffs and the incidence of goitre.

LITERATURE.

BLEYER, B. (1926). Zur kenntnis des Jods als biogenes Element. *Biochem. Zeitsch.*, No. 170, pp. 265-276.

BODNAR, J., AND STRAUB, J. (1930). Untersuchung des endemischen kropfes in Ungarn auf Gound der Jodmangeltheorie. *Biochem. Zeitsch.*, No. 227, pp. 237-244.

HERCUS, C. E., BENSON, W. N., AND CARTER, C. L. (1925). Endemic Goitre in New Zealand and its Relation to the Soil Iodine. *Jour. of Hyg.*, Vol. 24, pp. 321-402.

HERCUS, C. E., AND ROBERTS, K. C. (1927). The Iodine Content of Foods, Manures, and Animal Products in Relation to the Phophylaxis of Endemic Goitre in New Zealand. *Jour. of Hyg.*, Vol. 26, pp. 49-83.

HERCUS, C. E., *ET AL* (1931). Further Observations on the Occurrence of Iodine in Relation to Endemic Goitre in New Zealand and on Iodine Metabolism. *Jour. of Hyg.*, Vol. 31, pp. 493-522.

MAYRHOFER, A., SCHNEIDER, C., AND WASITZKY, A. (1932). Biochemische Studien über das Vorkommen kleiner Mengen von Jod und Fluor in Organismus. *Bioch. Zeit.*, No. 251, pp. 70-86.

McCLENDON, J. F. (1927). The Distribution of Iodine with Special Reference to Goitre. *Phys. Reviews*, Vol. 7, pp. 189-252.

ORR, J. B., AND LEITCH, I. (1929). Iodine in Nutrition. *Medical Research Council Report*, No. 123.

ORR, J. B. Private communication.

SCHARRER, K. (1928). Chemie und Biochemie des Jods.

SCHARRER, K., AND SCHWAIBOLD, J. (1928). Zur kenntnis des Jods als biogenes element. *Bioch. Zeit.*, No. 195, pp. 233-237.

SCHEFFER, L. (1932). Jodgehalt der Nahrung und Kropfhaüfigkeit. *Bioch. Zeit.*, No. 247, pp. 418-423.

VON FELLENBERG, TH. (1923). Untersuchungen über das Vorkommen von Jod in der Natur. *Bioch. Zeit.*, No. 139, p. 371.

VON FELLENBERG, TH. (1924, 1). Untersuchungen über das Vorkommen von Jod in der Natur. *Bioch. Zeit.*, No. 152, pp. 141-152.

VON FELLENBERG, TH. (1924, 2). Untersuchungen über das Vorkommen von Jod in der Natur. *Bioch. Zeit.*, No. 152, pp. 128-131.

VON FELLENBERG, TH. (1926). Das Vorkommen, der krieslauf und der stoffwechsel des Jods. *Ergebn. der Phys*, No. 25, pp. 176-363.

Studies in Mineral Metabolism XXX.

Variations in the Iodine Content of Grasses at different stages of Growth and a note on the Iodine Content of Milk.

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MANY anomalous results regarding the iodine content of plants are encountered in the literature. These cannot be attributed solely to experimental errors, and it seems quite feasible that, like some of the other more common elements, the iodine present in plants and vegetables may vary at different stages of growth.

Von Fellenberg (1926) reported on the seasonal variations in the iodine present in plants. He analysed the leaves obtained from *Helianthus tuberosus* at different times of the year. The leaves were collected on the 13th September, 4th December and 3rd March the following year, and the iodine contents expressed in γs per 100 grams dry weight were 12·5, 71·5 and 44·5 respectively. Similar variations were observed in the case of leaves from Beech trees. Leaves collected in May contained 1·3 γ, those collected in August, 13·8, while the former year's leaves contained 10·7 γ iodine per 100 grams calculated on the dry weight.

Prior to the commencement of the present study, experimental grass plots were established at Onderstepoort for another investigation. At the end of January, 1932, the grasses were firmly established and all the plots were cut and then allowed to grow freely. A portion of each plot was cut at monthly intervals so that samples were obtained representing the grass at stages of growth of 1, 2, 3, etc., up to 12 months. These samples were dried in the shade, finely ground and the iodine determined.

The rainfall in inches recorded at Onderstepoort for the twelve months was as follows:—

February	4·8	inches.
March	2·1	,,
April	0·8	,,
May	0·2	,,
June	nil	,,
July	nil	,,
August	nil	,,
September	0·9	,,
October	1·8	,,
November	2·6	,,
December	3·5	,,
January	1·5	,,

In the following tables descriptions of the samples as well as their iodine contents at different stages of growth are given.

TABLE I.

Species: *Panicum maximum.*

Stage of Growth in Months.	Description of Sample.	Iodine Content Expressed as γ % on the Dry Weight.
1	Green, with flower heads	26·5
2	Green, seeds falling out	26
3	Mixed, mainly green, seeds falling out	14·5
4	Mixed, seeds fallen out	15
5	Mixed, mainly brown, seeds fallen out	16
6	Brown, seeds fallen out	16·6
7	Brown, seeds fallen out	21
8	Mixed, mainly brown, seeds fallen out	31
9	Mixed, new flower heads present	20
10	Mixed, mainly green, new flower heads present	22·4
11	Mixed, mainly green, with flower heads	12
12	Mixed, mainly green, seeds falling out	16

TABLE II.

Species: *Themedra triandra.*

Stage of Growth in Months.	Description of Sample.	Iodine Content Expressed as γ % on the Dry Weight.
1	Green, with flower heads	14
2	Green, with flower heads	14
3	Mixed, mainly green, seeds falling out	10·5
4	Mixed, seeds falling out	18·5
5	Mixed, mainly brown, seeds falling out	25
6	Brown, seeds fallen out	23
7	Brown, seeds fallen out	26
8	Mixed, mainly brown, seeds fallen out	51
9	Mixed, mainly brown, new flower heads present	35
10	Mixed, new flower heads present	37
11	Mixed, mainly green, with flower heads	20·5
12	Mixed, with flower heads	16

TABLE III.
Species: *Rhynchelythrum roseum*.

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads...............................	20
2	Green, seeds falling out...............................	16·5
3	Mixed, mainly green, seeds falling out.................	9
4	Mixed, seeds falling out...............................	16
5	Brown, seeds fallen out................................	19
6	Brown, seeds fallen out................................	25
7	Brown, seeds fallen out................................	32
8	Mixed, mainly brown, seeds fallen out..................	35
9	Mixed, mainly brown, new flower heads present..........	39
10	Mixed, mainly brown, new flower heads present..........	22
11	Mixed, mainly brown, with flower heads.................	33
12	Mixed, mainly brown, seeds falling out.................	28·5

TABLE IV.
Species: *Cymbopogon plurinodis*.

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads...............................	15
2	Green, seeds falling out...............................	13
3	Mixed, mainly green, seeds falling out.................	12
4	Mixed, mainly brown, seeds falling out.................	22
5	Mixed, mainly brown, seeds falling out.................	30·5
6	Brown, seeds falling out...............................	36
7	Brown, seeds falling out...............................	22·5
8	Mixed, mainly brown, seeds fallen out..................	31
9	Mixed, mainly brown, seeds fallen out..................	16
10	Mixed, new flower heads present........................	24·5
11	Mixed, with flower heads...............................	28·5
12	Mixed, mainly green, with flower heads.................	8

TABLE V.
Species: *Urochloa pullulans*.

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads { Stalks........................	8·2
	{ Leaves.......................	11
2	Green, seeds falling out...............................	8
3	Mixed, mainly green, seeds falling out.................	6
4	Brown, seeds fallen out................................	14
5	Brown, seeds fallen out................................	16
6	Brown, seeds fallen out................................	17
7	Brown, seeds fallen out................................	20·5
8	Mixed, mainly brown, seeds fallen out..................	24·5
9	Mixed, seeds fallen out................................	33
10	Mixed, new flower heads present........................	30
11	Mixed, with flower heads...............................	21
12	Mixed, mainly green, seeds falling out.................	21·5

The monthly samples obtained from three other plots were divided into stalks, leaves, and tops. The tops represented the flower heads at different stages of development and as these usually formed only a small fraction of the total weight of the samples, iodine determinations were carried out on only a few of these flower heads.

TABLE VI.

Species: *Hyparrhenia hirta.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads............	Stalks...	90	4·2
		Leaves..	200	11
		Flowers..	17	24
2	Green, with flower heads............	Stalks...	740	3·8
		Leaves..	540	14
		Flowers..	202	29
3	Green, mainly green, seeds falling out	Stalks...	720	Nil.
		Leaves..	620	17
4	Mixed, seeds falling out............	Stalks...	1,015	Nil.
		Leaves..	512	20·5
5	Mixed, mainly brown, seeds falling out	Stalks...	905	6·2
		Leaves..	543	26·5
6	Brown, seeds fallen out............	Stalks...	788	6
		Leaves..	490	28
7	Brown, seeds fallen out............	Stalks...	1,010	4
		Leaves..	590	28
8	Mixed, mainly brown, seeds fallen out	Stalks...	620	10
		Leaves..	415	28
9	Mixed, mainly brown, new flower heads present	Stalks...	420	11·5
		Leaves..	240	34·6
10	Mixed, new flower heads present....	Stalks...	745	10·6
		Leaves..	415	41
11	Mixed, with flower heads............	Stalks...	925	12
		Leaves..	875	26
		Flowers..	300	34·6
12	Mixed, seeds falling out............	Stalks...	1,567	12
		Leaves..	1,155	28
		Flowers..	577	16

TABLE VII.

Species : *Amphilophis insculpta.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads............	Leaves..	330	10·8
2	Green, seeds falling out.............	Stalks... Leaves.. Flowers..	290 380 90	Nil. Trace. 15·2
3	Mixed, mainly green, seeds falling out	Stalks... Leaves..	420 570	Nil. Trace.
4	Mixed, mainly brown, seeds fallen out	Stalks... Leaves..	565 572	6·5 9
5	Brown, seeds fallen out.............	Stalks... Leaves..	516 439	6·5 11
6	Brown, seeds fallen out.............	Stalks... Leaves..	290 305	8·6 25·6
7	Brown, seeds fallen out.............	Stalks... Leaves..	500 486	19·6 32·6
8	Mixed, mainly brown, seed fallen out	Stalks... Leaves..	270 185	11·0 40
9	Mixed, mainly brown, new flower heads present....................	Stalks... Leaves..	— —	7·0 40
10	Mixed, new flower heads present.....	Stalks... Leaves..	400 425	16 36
11	Mixed, mainly green, with flower heads	Stalks... Leaves..	600 1,290	22 33
12	Mixed, mainly green, seeds falling out	Stalks... Leaves..	1,260 1,674	6·5 26

TABLE VIII.

Species: *Eragrostis superba.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads...........	Leaves..	—	11·3
2	Green, with flower heads...........	Total.... Sample	—	8
3	Mixed, mainly green, seeds falling out	Stalks... Leaves.. Flowers..	200 180 90	Trace. 12·6 8
4	Mixed, seeds falling out............	Stalks... Leaves..	559 340	4 10
5	Mixed, mainly brown, seeds falling out	Stalks... Leaves..	365 320	6 9
6	Mixed, mainly brown, seeds fallen out	Stalks... Leaves..	370 230	6 18
7	Brown, seeds fallen out............	Stalks... Leaves..	450 171	6 16
8	Mixed, mainly brown, seeds fallen out	Stalks... Leaves..	335 275	15 31
9	Mixed, mainly brown, new flower heads present	Stalks... Leaves..	319 355	15 27
10	Mixed, new flower heads present.....	Stalks... Leaves..	117 395	20 29
11	Mixed, mainly green, with flower heads	Stalks... Leaves..	957 1,056	11·5 18
12	Mixed, seeds falling out............	Stalks... Leaves..	2,280 2,760	11·5 13·5

Graphical representations of the results (figures I, II and III) show conclusively that there is an appreciable difference in the iodine content of the same species of grass cut from the same plot at different stages of growth. It is interesting to note that at the end of the third month, i.e. when the grass is turning brown, the iodine content is at a minimum. From then onwards there is a progressive increase, the amount of which varies for the different grasses analysed. In some cases this increase is of such an order that the iodine in the samples cut from four months onwards is higher than that of the first month. This fact appears to be very important for, as stated further on, several investigators find that the iodine content of thyroids shows seasonal variations which may bear some relation to the seasonable iodine content of the pasture consumed.

Stage of Growth in Months.
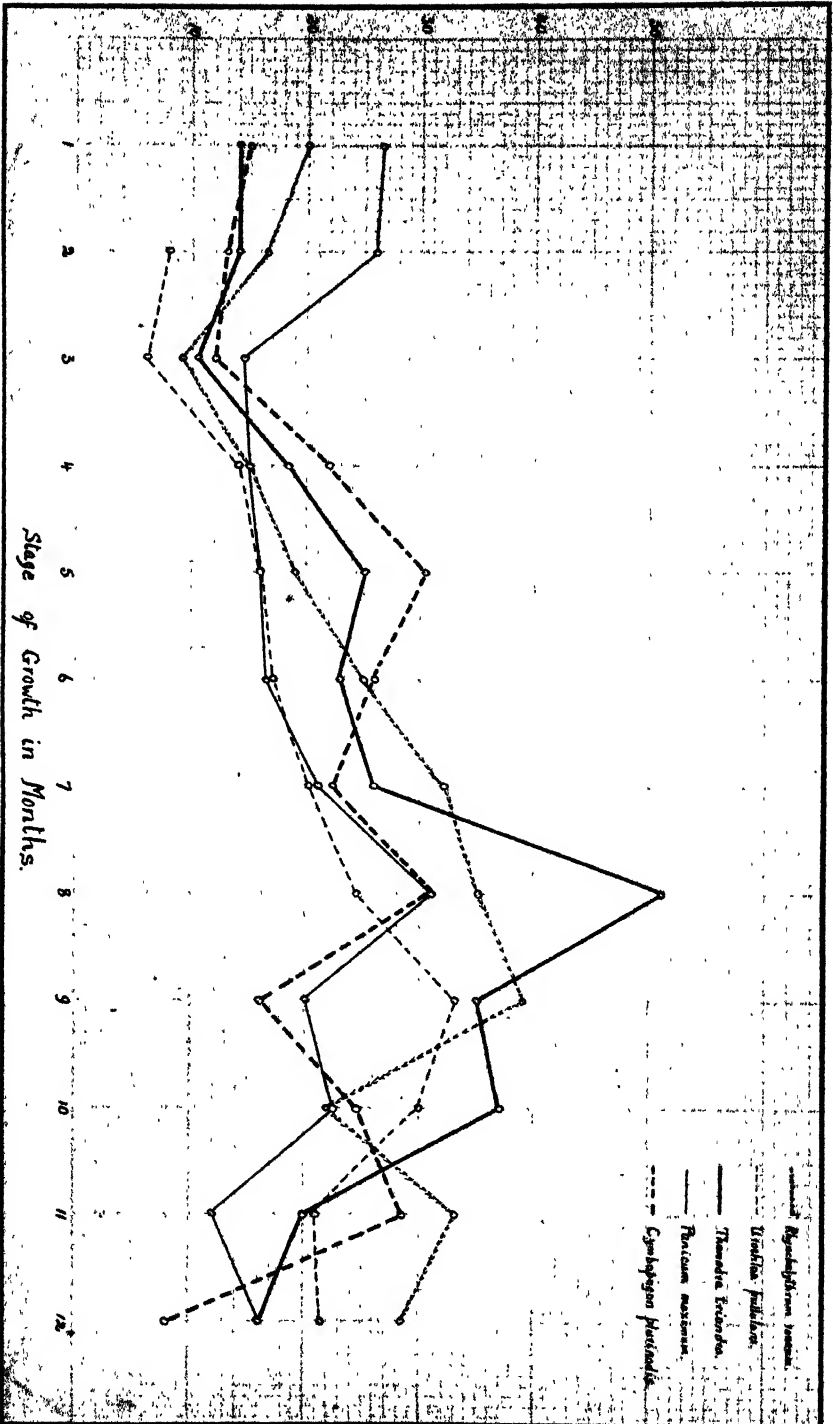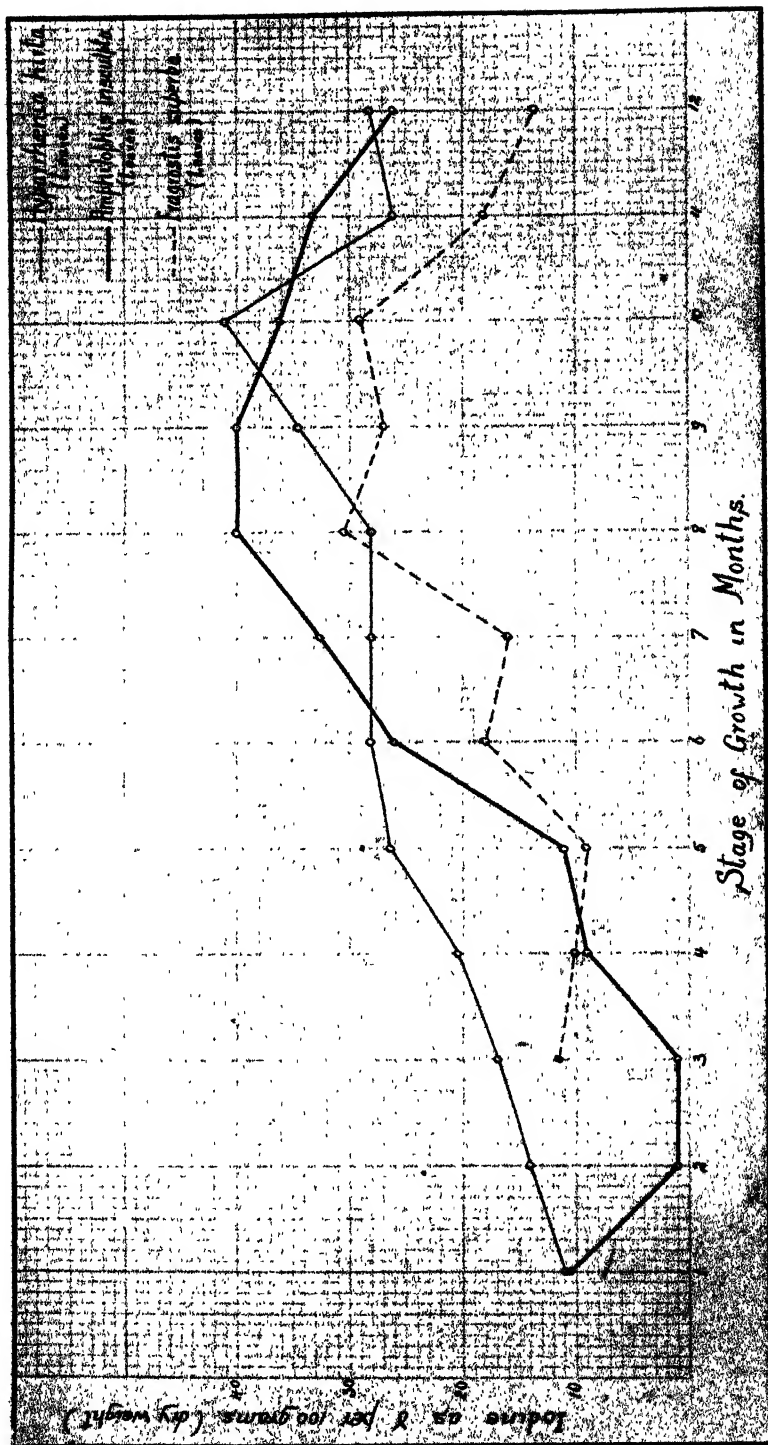
Fig. I.

I. J. B. Blom.

FIG. II.

Fig. III.

Fig. III.
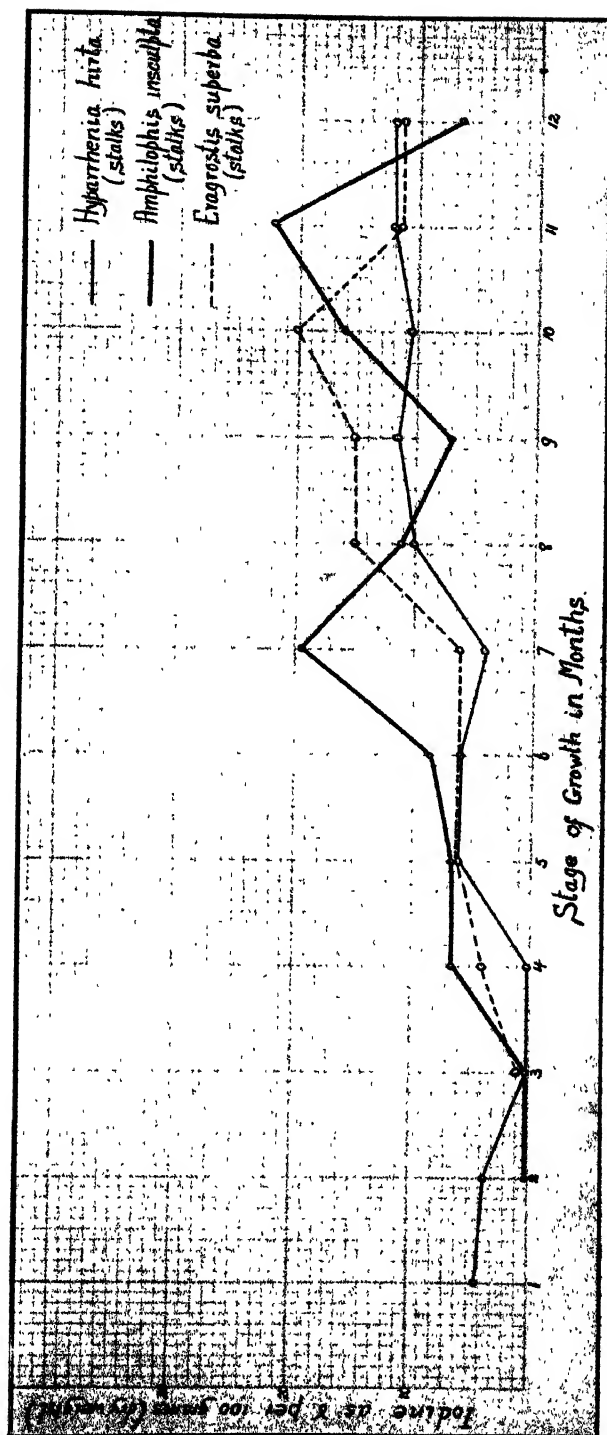
After the first rains in the spring there is a stage of active growth and the iodine content reaches a maximum about the eighth or ninth month. Beyond this stage the iodine content fluctuates but, in general, the trend is downwards so that at the end of the test, i.e. after twelve months the iodine is practically at the same level as at the end of the first month. The wide variations in the iodine content of grasses at different stages of growth are well worth recording. The iodine present in the composite sample is made up of that present in the various constituents, i.e. the stalks, leaves, and flowers or seeds or whatever remains after the seeds have fallen out. The weights as well as the iodine content of the different fractions vary from month to month and consequently the iodine present in the sample will vary accordingly. The results of the three grasses divided into fractions show that the leaves and flowers contain more iodine than the stalks. Hence the increase of iodine content during the winter would depend in some measure on the relative quantity of leaves as compared with stalks in the stand of grass.

The results are in close agreement with those of von Fellenberg on beech leaves. Hercus and Roberts (1927) in studying the seasonal variations in the iodine content of vegetable matter reported that the iodine concentration was at a maximum in autumn and winter and a similar increase in the autumn has been observed by Orr and Leitch (1929).

It has also been established by Orr and others that the iodine content of the thyroid can be raised by the administration of small doses of iodine. Furthermore, it was shown by Martin (1912, 1913) and confirmed by Seidell and Fenger (1912, 1913) that the iodine of sheep thyriods showed a regular variation with a maximum in autumn and a minimum in spring. Orr naturally assumes that this is due to the variations in the amount of iodine present in the pasture.

Dawbarn and Farr (1932) in a survey of the thyroids of about 700 sheep in Australia found results in close agreement with those of Martin and others. Further evidence for the assumption of Orr was obtained by these authors who found that after a period of severe drought the iodine concentration in the thyroid was much higher than during or after a good season.

The wide variations in the iodine content of grass collected at different stages of growth and the consequent seasonable variations in the iodine concentration in the thyroid suggest that similar variations might be encountered in the case of foodstuffs. This has been established by Hercus and Roberts (1927) who obtained figures for beef ranging from 1·2 to 80 γ per 100 gm. during a period of 9 months and the iodine content of eggs was 6·4, 16·0 and 29 γ per 100 gm. respectively for three consecutive months. In order to obtain comparative values for the iodine content of foodstuffs, samples for analysis should be collected at the same time and at the same stage of growth or development.

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THE IODINE CONTENT OF MILK.

During the course of the present investigation iodine analysis were carried out from time to time on milk from cows in a nutrition experiment (du Toit, Malan and Groenewald). All the cows were fed on the same diet with the addition of certain mineral salts, all with either a trace or only a minute quantity of iodine in the form of an impurity. In addition to this bovine 3,677 was given 0·1 gm. potassium iodine daily for a period of three years. The results are recorded in Table IX. The effect of the administration of iodine to bovine 3,677 is reflected in the high iodine content of its milk.

TABLE IX.

Bovine No.	Date of Sampling.	Time of Sampling.	Iodine Expressed as γ per 100 c.c. Milk.
3640	24.11.32	Afternoon	4·6
3640	25.11.32	Morning	5·1
3643	25.11.32	Morning	5·9
3645	24.11.32	Afternoon	4·6
3645	25.11.32	Morning	8·4
3649	24.11.32	Afternoon	5·5
3649	25.11.32	Morning	1·7
3655	24·11·32	Afternoon	5·5
3655	25.11.32	Morning	2·5
3642	21.11.23	Afternoon	4·5
3642	22.11.32	Morning	3·1
3642	22.11.32	Afternoon	2·6
3653	21.11.32	Afternoon	6·7
3653	22.11.32	Morning	6·7
3653	22·11·32	Afternoon	1·7
3677	24.11.32	Afternoon	107·0
3677	25·11·32	Morning	51·3

Another case worth recording is that of bovine 3643. This cow was treated for metritis and the uterus was douched with Lygol's iodine on the 10th, 17th and 20th October, 1932. The iodine content of the milk sampled after the treatment is given in Table X.

TABLE X.

Iodine Content of Milk from bovine 3643.

Date of Sampling.	Time of Sampling.	Iodine as γ per 100 c.c. Milk.
24.10.32	Afternoon	121·0
25.10.32	Morning	120·0
31.10.32	Morning	70·4
31.10.32	Afternoon	49·0
4.11.32	Afternoon	9·6
5.11.32	Morning	17·4
18.11.32	Afternoon	6·7
21.11.32	Morning	4·5

The non-protein portion of milk was next considered. The proteins were precipitated by the addition of trichloracetic acid to the milk and iodine was determined in the filtrate. These results, together with the total iodine present in the milk, are given in Table XI.

TABLE XI.

Bovine No.	Date of Sampling.	Time of Sampling.	Iodine Expressed as γ per 100 c.c. Milk.	
			Iodine Present in Filtrate.	Total Iodine.
3677......	19.10.32...........	Morning.............	29·6	99
3677......	19.10.32	Afternoon.............	15·2	62
3677......	21.10.32..	Morning..............	17·0	64
3677......	21.10.32....	Afternoon..	18·8	83
3677......	22.10.32....	Morning....	28·6	86
3677......	24.10.32....	Afternoon.............	31·0	39
3677......	25.10.32....	Morning.........	31·0	45
3677......	27.10.32....	Afternoon....	39·2	59
3677.. ...	28.10.32....	Morning.....	24·5	59
3642......	24.10.32....	Afternoon....	121·0	121·0
3642... ..	25.10.32..	Morning...	119·0	120·0
3640......	24.10.32....	Afternoon....	2·7	5·6
3649.....	24.10.32....	Afternoon..	3·7	9·2
3655... ..	24.10.32.............	Afternoon.	4·3	8·6

It appears that in some cases all the iodine in milk is present in the non-protein portion but in others only a fraction of the total iodine is present in the filtrate and some of the iodine must be associated either mechanically or chemically with the proteins which are precipitated.

LITERATURE.

DAWBARN, MARY C., AND FARR, F. C. (1932). Variations in the Dry Weight and Iodine Content of the Thyroid Glands of Sheep under Uniform and Varying Conditions. *Australian Jour. of Exp. Biol. and Med. Sc.*, Vol. 10, pp. 119-142.

HERCUS, C. E., AND ROBERTS, K. C. (1927). The Iodine Content of Foods, Manures, and Animal Products in Relation to the Phrophylaxis of Endemic Goitre in New Zealand. *Journ. of Hyg.*, Vol. 26, pp. 49-83.

MALAN, A. I., DU TOIT, P. J., AND GROENEWALD, J. W. Minimum mineral requirements of Cattle. To be published in the next issue of this Journal.

MARTIN (1912, 1913). Quoted by Orr and Leitch, 1929.

ORR, J. B., AND LEITCH, I. (1929). Iodine in Nutrition. *Medical Research Council Report*, No. 123.

SEIDELL, A., AND FENGER, F. (1912-1913). Seasonal Variations in the Iodine Content of the Thyroid Gland. *Jour. Biol. Chem.*, Vol. 13, pp. 517-526.

VON FELLENBERG, TH. (1926). Das Vorkommen der krieslauf und der Stoffwechsel des Jods. *Ergebn. der Phys*, No. 25, pp. 176-363.

Section V.

Chemical Blood Studies.

Chemical Blood Studies.

VI. A Serial Study over a 12 Month Period of Some Organic Constituents in "Laked" and "Unlaked" Blood Filtrates of Healthy Sheep (Merino) of Various Ages.*

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F. General Summary.

G. Acknowledgements.

A. INTRODUCTION.

IN view of the fact that there are no figures available for most of the organic constituents of blood for any of the domestic animals under South African conditions and relatively few data from other countries, this research work was initiated.

Many of the figures recorded in the literature are of little value, since they were obtained from a small number of animals, examined only a very few times and because as a rule no information as regards breed, diet, environment, etc., are stated. Furthermore, the period over which blood was withdrawn was short—no serial analyses over any length of period (say one year) having been found recorded. Also quite a number of figures present analyses of blood collected at slaughter houses and analysed after varying periods, usually several hours after withdrawal. Owing to the instability of most of the blood constituents such figures must be regarded with caution.

Since South African conditions vary greatly as compared with those of other countries in regard to variable rainfall, periodic droughts, and other climatic factors, these figures will be of considerable value for comparative purposes, particularly as the same animals were repeatedly bled during the various seasons.

It may be stated that this experiment was carried out during a period of exceptional drought, the rainfall being the minimum for the last 30 years for this area, and this may have influenced to some degree the composition of the blood. Of the influence of humidity and external temperature on composition practically nothing is known.

In this series various other articles (Graf, 1933) dealing with different infectious diseases of stock have been published, and it was felt that accurate "normal" data were imperative for the purposes of evaluating the data in respect to what could be regarded as "pathological" and what as "normal". In the articles referred to, analyses of blood were undertaken before infection in order to supply a comparative basis, but, if once "normal ranges" for the various blood constituents have been determined, this procedure would largely become redundant. Analyses could then be undertaken at any time during the course of a disease and compared with the "normal range" for any particular type of animal. In this article, however, only the composition of sheep's blood will be considered.

The main purpose of the data is therefore to give such figures of healthy Merino sheep, under stated conditions as to provide a comparative basis for pathological conditions. A fixed ration (except for an allowance of green feed during the summer) was adhered to throughout, except where otherwise stated.

The following constituents have been determined: haemoglobin (Hb.), sugar, total nitrogen (T.N.), non-protein nitrogen (N.P.N.), urea nitrogen (U.N.), total creatinine nitrogen (T.C.N.), uric acid nitrogen (U.A.N.), and amino-acid nitrogen (A.A.N.).

It is intended to continue with the determination of other constituents like cholesterol, pigments, ammonia ,lactic acid, etc., also physico-chemical determinations such as viscosity, hydrogen ion concentration, sedimentation rate of cellular elements, etc., in order to obtain as extensive information as possible on the normal composition and physical properties of the blood of domestic animals.

Five groups, arranged according to age and sex, were examined.

The analyses were done over a period of 15 months in the case of adult sheep, and 11 months in the case of lambs, figures over a relatively long period of time being thus obtained.

A satisfactory method for the preparation of " unlaked " blood filtrates was published by Folin (1930), and because of the following reasons, such filtrates together with the usual " laked " blood filtrates were analysed :

(1) To acquire normal figures for both filtrates in order always to be able to compare them with both " laked " and " unlaked " figures published by other research workers.

(2) For comparing " laked " with " unlaked ", since there may be great differences in the concentration of the constituents, in these two filtrates. This difference may be wholly normal or possibly of pathological significance (e.g. the difference between " laked " and " unlaked " blood will not only be a result of the presence of disintegration products of the cells which may be formed during the dissolution of the cell, but also as a direct result of the constitution of the cells as far as concentration of the different constituents in the plasma and cell are concerned. In the case of the urea only a small difference between the " laked " and " unlaked " blood is noted and the view that the urea concentration in cells and plasma are about the same, is thus strengthened. On the other hand, a difference of about 19 per cent. is noted in the sugar concentration in " laked " and " unlaked " filtrates, " laked " containing the higher figure. The explanation can only be ascribed to two factors viz. : (a) that through the laking of the cells substances which may react with the sugar reagent are liberated, and (b) that the actual concentration of sugar is higher in the cells. If (1) is not taken into consideration it would be possible to calculate the percentage sugar in the cells, provided, of course, that the cell volume is known.)

(3) The " unlaked " blood analysis may prove to be more valuable, as more information becomes available than the " laked " blood analysis, Wu (1932), Folin and Svedberg (1930).

B. TECHNIQUE.

(a) ARRANGEMENT OF GROUPS (A-E).

Twenty-three sheep were classified according to age and sex into the following groups : —

Group A.—Six adult ewes. (Of this group one died during the experiment, 23.9.32 S. 24163. Two more sheep were excluded from the experiment after 6 months: S. 24156 and S. 24160).

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Group B.—Three young lambs born of the above ewes during the experiment (one excluded after 6 months, S. 33589).

Group C.—Three six-tooth ewes. Two excluded after 6 months, S. 22204, S. 25142).

Group D.—Six ewe lambs (one excluded after 6 months, S. 29471).

Group E.—Five six-tooth wethers (two excluded after 6 months: S. 31662 and S. 31905).

In order to keep the environmental conditions the same for all groups, all these sheep were permitted to run in the same camp and access to the same feed troughs and water supply.

(b) ANTHELMINTHIC TREATMENT.

All the sheep, except of course the lambs of group B, were examined for worms (through the courtesy of Dr. H. O. Mönnig) and showed only a very slight infection, chiefly of wireworm (*Haemonchus contortus*). Once every month the animals were dosed with Government Wireworm Remedy (see also 1st paper of this series).

Length of Wool.—At the start of the experiment the wool was ½ inch long. All the sheep except the three young lambs were shorn on the 8th February, 1932.

Environment.—The sheep were kept throughout in the same pen sufficiently large to permit of ample exercise. Shelter was available to provide protection against the sun, wind, cold and rain.

Rations.—The following ration was supplied: 1 lb. crushed mealies per sheep and dry grass *ad lib.*, were supplied at 6.30 a.m. Also dry grass *ad lib.*, at 4.30 p.m.; green fodder *ad lib.* (whenever available during the summer months), except from 24th December, 1932, to the end of the experiment. In addition, one oz. of salt per sheep was provided on Thursdays.

It was not considered of sufficient value to determine accurately the intake of food by each individual animal, since the present work is not so much concerned with how the food influences the blood composition, but rather a study of the blood composition on a relatively fixed diet, as was pointed out before to provide normal figures as a basis for pathological studies. This system also furthermore permits of seasonal variations in the normal composition of the blood, whether due to diet, environmental temperature, humidity, etc., or not, to be taken into consideration. It, therefore, allows for an accurate comparison of the figures obtained for the various constituents in health and during any particular infection.

The weights of the animals recorded below their respective tables of data give a clear indication that the animals were in good condition and progressed normally as far as increase in weight is concerned. The food supplied was above a mere maintenance ration.

In order to have a further check on the state of health of these sheep, they were daily temperatured, and in no case was an abnormal reaction encountered.

(c) METHODS OF CHEMICAL ANALYSES.

All the methods utilized and any modifications that were deemed advantageous have been fully recorded in the first paper of this series (Chemical Blood Studies I. See under " References ").

(d) ARRANGEMENT OF EXPERIMENTAL DATA.

Complete tables of analytical data (" laked " and " unlaked ") for every sheep in a group together with the weights and history are given for each group, followed by a discussion of every constituent for that particular group.

The urea (46·66 per cent. N) uric acid (33·3 per cent. N) and the " total " creatinine nitrogen (37 per cent. N) are expressed both as " N " and as such. The lowest column on the tables is the rest nitrogen, which was determined by subtracting from the non-protein nitrogen the urea nitrogen, amino acid nitrogen, uric acid nitrogen, and the " total " creatinine nitrogen. As the rest-nitrogen is obtained by calculation, the figures are obviously not absolute, but are influenced by the limits of experimental error of the various fractions.

In all five groups this arrangement is adhered to. Thereafter a summary and comparison of the combined groups is succeeded by tables showing a comparison of these data with those obtained by other workers.

Use is made of graphs to elucidate some points (Graph I, p. 165; II, p. 178; III, p. 186; IV, p. 187).

The following abbreviations have been used, both in the tables, in the text, and on the graphs :—

L = " laked ".
U = " unlaked ".
S = sheep.
Hb = haemoglobin.
T.N. = Total nitrogen.
N.P.N. = Non-protein nitrogen.
U.N. = Urea nitrogen.
T.C.N. = " Total " creatinine nitrogen.
U.A.N. = Uric acid nitrogen.
A.A.N. = Amino-acid nitrogen.
R.N. = Rest-nitrogen.
Av. = Average.
t.l. = too low to be determined colorimetrically.
No. = number.
detms. = determinations.
diff. = difference.
" L ".A. = " laked " group A.
" L ".B. = " laked " group B.
" U ".A. = " unlaked " group A.
" U ".B. = " unlaked " group B.
anal. and an = analysis.

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C. **EXPERIMENTAL DATA.**

(*a*) GROUP A, TABLES (1-17), GRAPH I AND DISCUSSION OF GROUP A.

Sheep No. 15398, Table 1.
,, No. 24136, Table 2.
,, No. 24156, Table 3.
,, No. 24158, Table 4.
,, No. 24160, Table 5.
,, No. 24163, Table 6.

TABLE 1.—Sheep 15398.

Date.		4th Nov., 1931.	6th Nov., 1931.	2nd Dec., 1931.	10th Dec., 1931.	10th March, 1932.	12th April, 1932.	19th April, 1932.	22nd April, 1932.	27th April, 1932.	4th July, 1932.	8th July, 1932.	30th Sept., 1932.	15th Dec., 1932.	22nd Dec., 1932.	24th Jan., 1933.	27th Jan., 1933.
Haemoglobin, gm. per 100 c.c.		16·54	14·72	15·42	14·28	13·87	12·28	12·42	13·87	14·95	17·51	17·18	13·31	16·29	12·94	13·87	13·68
Sugar, mg. % (Glucose)	L	42·74	54·05	51·81	38·17	48·54	43·29	—	45·45	47·62	47·39	39·06	38·61	50·76	47·39	43·10	46·87
	U	31·90	41·66	37·04	33·67	40·32	36·10	—	31·75	39·22	34·25	34·01	29·41	40·00	32·15	38·17	31·25
Total-N., gm. N %		2·842	2·896	2·996	2·856	2·926	2·926	2·856	2·828	3·093	3·234	3·094	3·024	2·807	2·758	2·877	2·786
Non-Protein Nitrogen, mg. N %	L	21·72	23·36	30·0	30·0	27·78	19·23	21·82	18·63	24·29	23·54	17·44	19·23	42·54	45·46	26·78	22·22
	U	15·79	14·63	26·55	22·06	17·14	10·94	12·82	11·15	16·48	12·50	10·71	15·00	33·88	39·46	16·76	14·02
Coaguable Nitrogen, gm. N %	L	2·820	2·875	2·960	2·826	2·898	2·907	2·834	2·809	2·979	3·210	3·077	3·005	2·764	2·712	2·850	2·764
	U	2·826	2·883	2·963	2·834	2·909	2·915	2·843	2·817	2·987	3·221	3·083	3·009	2·773	2·719	2·860	2·772
Urea. mg. N %	L	10·11	11·42	18·61	11·72	5·66	4·65	5·49	4·33	4·56	3·83	3·35	3·44	18·51	31·53	6·69	3·60
mg. U %	L	21·21	23·94	39·08	24·60	12·80	9·70	11·50	9·50	9·50	8·00	7·00	7·14	38·85	66·15	14·07	7·56
mg. N %	U	10·49	11·20	17·02	11·90	7·51	4·71	5·41	4·79	4·48	3·65	t 1	3·76	15·53	31·53	5·49	3·35
mg. U %	U	22·00	23·52	35·70	24·99	15·75	9·87	11·34	10·00	9·40	7·66	t 1	7·98	32·55	66·15	11·55	7·14
"Total," -Creatinine. mg. N %	L	1·94	1·86	2·66	2·54	2·23	1·97	1·00	2·05	2·23	2·01	2·23	2·04	2·36	2·36	2·66	2·11
mg. TC %	L	5·24	5·02	7·20	6·06	6·00	5·32	5·14	5·34	6·00	5·40	6·00	5·50	6·36	6·36	7·20	5·68
mg. N %	U	1·60	1·44	2·08	2·14	1·82	1·82	1·49	1·56	1·49	1·49	1·38	1·71	1·90	1·90	1·82	1·67
mg. TC %	U	4·32	3·86	5·64	5·76	4·90	4·00	4·00	4·22	4·00	4·00	3·72	4·60	5·14	5·14	4·90	4·50
Uric acid. mg. N %	L	·44	·24	·44	·39	·45	·33	·23	·21	·33	·38	·33	·27	·18	·39	·35	·39
mg. UA %	L	1·33	·71	1·31	1·18	1·36	1·00	·69	·64	1·00	1·16	1·00	·82	·55	1·18	1·04	1·18
mg. N %	U	—	—	·30	·13	·18	·15	·13	·12	—	·16	·14	·08	·13	·16	·22	·18
mg. UA %	U	—	—	·91	·38	·53	·44	·40	·35	—	·48	·41	·25	·41	·47	·66	·53
Amino-Acid, mg. N %	L	5·56	5·18	7·18	7·00	7·53	7·00	8·75	6·36	8·24	5·43	4·93	6·73	6·03	7·00	7·00	6·36
	U	4·52	4·24	6·36	5·96	5·18	4·12	4·67	4·67	5·60	4·38	3·41	5·42	3·61	4·73	5·15	4·21
Rest Nitrogen, mg. N %	L	3·67	4·66	1·11	8·36	11·91	5·88	5·45	5·68	8·84	11·89	6·60	6·75	14·46	3·18	10·08	8·71
	U	·82*	2·25*	·79	1·93	2·45	0·47	1·12	·01	4·91*	2·82	5·78	4·03	11·71	0·14	4·08	4·61

History : The sheep had bluetongue in September, 1926.

* Includes uric acid N.

Weights : 18.12.31 — 116 lb.
22.1.32 — 136† lb.
8.2.32 — lambed, lamb No. 33589.
23.2.32 — 110 lb.
29.4.32 — 105 lb.
25.5.32 — 111 lb.
22.7.32 — 122 lb.
24.8.32 — 130 lb.
17.10.32 — lambed.
29.11.32 — 104 lb.

TABLE 2.—Sheep 24136.

Date.		11th Nov., 1931.	13th Nov., 1931.	17th Dec., 1931.	18th Dec., 1931.	22nd March, 1932.	30th March, 1932.	12th April, 1932.	22nd April, 1932.	27th April, 1932.	17th May, 1932.	19th May, 1932.	9th June, 1932.	28th June, 1932.	29th June, 1932.	30th Sept., 1932.	15th Dec., 1932.	28th Jan., 1933.	30th Jan., 1933.
Haemoglobin, gm. per 100 c.c.		12·42	12·13	14·95	17·18	13·31	12·59	12·59	14·95	14·49	14·28	13·12	14·95	14·08	11·82	10·99	15·42	12·28	12·79
Sugar, mg. %. (glucose)	L	30·21	31·95	26·98	36·50	40·09	42·19	37·45	40·82	42·55	33·33	34·84	42·01	42·74	56·18	43·29	42·01	35·97	33·90
	U	25·90	27·80	19·61	25·00	34·72	34·97	33·11	32·36	35·59	30·49	31·15	35·46	34·25	51·28	40·00	35·46	32·62	29·07
Total-N., gm. N %.		2·716	2·660	2·702	2·709	2·842	2·772	2·758	2·877	2·898	2·961	2·870	2·975	2·828	2·694	2·485	2·632	2·632	2·562
Non-protein N. mgm. %.	L	17·65	32·78	28·56	23·24	20·14	16·39	18·07	17·14	17·04	15·15	16·04	—	14·28	22·72	17·14	37·50	21·58	21·96
	U	11·77	27·78	20·49	15·80	13·45	11·41	14·56	14·28	12·29	10·94	11·71	—	9·37	12·60	12·45	31·58	14·56	16·58
Coagulable Nitrogen. gm. N %.	L	2·698	2·627	2·773	2·686	2·822	2·756	2·740	2·860	2·881	2·946	2·854	—	2·814	2·651	2·468	2·594	2·611	2·540
	U	2·704	2·632	2·782	2·693	2·829	2·761	2·743	2·863	2·886	2·950	2·858	—	2·819	2·661	2·473	2·601	2·617	2·545
Urea. mg. N %.	L	3·69	17·69	13·04	8·34	7·58	4·32	6·71	7·09	5·71	3·27	3·35	—	2·64	3·65	3·55	15·95	6·24	7·70
mg. U %.	L	7·6	37·0	27·3	17·5	15·8	9·0	14·0	14·7	11·97	6·8	7·0	—	5·5	7·6	7·40	33·69	13·02	16·17
mg. N %.	U	3·83	17·61	13·29	8·75	7·87	4·81	6·94	7·00	5·35	3·35	2·84	—	1·00	4·12	3·65	15·95	6·24	7·33
mg. U %.	U	8·0	36·96	27·8	18·3	15·4	10·08	14·5	14·7	11·2	7·0	6·0	—	2·1	8·6	7·6	33·69	13·02	15·33
"Total" Creatinine. mg. N %.	L	2·55	2·48	2·55	—	2·06	2·06	2·06	1·49	2·01	1·86	1·75	1·89	2·11	3·34	2·29	2·01	1·64	1·89
mg. TC %.	L	6·96	6·70	6·86	—	5·54	5·54	5·54	4·0	5·40	5·02	4·70	5·14	5·68	9·00	6·16	5·40	4·40	5·14
mg. N %.	U	1·82	2·04	2·23	—	1·41	1·56	1·52	1·40	1·41	·95	1·49	1·56	1·41	2·11	2·01	1·89	1·37	1·67
mg. TC %.	U	4·90	5·54	6·00	—	3·78	4·22	4·10	3·78	3·80	2·56	4·00	4·16	3·80	5·68	5·40	5·11	3·72	4·50
Uric acid. mg. N %.	L	—	·18	·18	·21	·17	·23	·14	—	·19	·17	·39	·19	·22	—	·22	—	·16	·20
mg. UA %.	L	—	0·53	0·55	0·64	0·52	0·68	0·42	—	0·57	·53	1·18	·58	·65	—	·67	—	·47	·61
mg. N %.	U	—	·16	—	—	·08	·13	—	—	—	·12	·14	·18	·16	—	·08	—	·10	·09
mg. UA %.	U	—	0·47	—	—	0·25	0·39	—	—	—	·35	·43	·55	·47	—	·23	—	·31	·28
Amino-acid, mg. N %.	L	4·52	4·83	5·83	5·60	5·47	5·83	5·83	5·83	5·60	5·0	4·83	4·83	3·68	5·71	4·67	5·07	5·00	5·38
	U	3·90	4·67	4·12	4·83	4·67	4·67	4·52	5·00	5·38	4·12	3·68	3·18	2·98	4·36	4·35	3·68	4·54	5·07
Rest Nitrogen, mg. N %.	L	6·89*	7·25	6·86	9·09†	4·86	3·95	3·33	2·82*	3·53	4·85	5·72	—	5·63	10·02*	6·41	13·47*	8·54	6·79
	U	2·22*	2·99	·85*	2·22†	·08	·24	1·58*	0·88*	0·15*	2·40	3·56	—	3·92	3·01*	2·36	9·06*†	3·21	2·42

* Includes Uric acid N.
† Includes "T" Creatinine N.

History: The sheep had bluetongue in June, 1929.
Weights: 18.12.31 — 94 lb. lambed, 29.12.31. lamb No. 33205.
23. 2.32 — 85 lb.
29. 4.32 — 91 lb.
26. 5.32 — 90 lb.
22. 7.32 — 92 lb.
24. 8.32 — 96 lb.
13.10.32 — lambed.
29.12.32 — 85 lb.

TABLE 3.—Sheep 24156.

Date.		4th Nov., 1931.	6th Nov., 1931.	2nd Dec., 1931.	10th Dec., 1931.	12th April, 1932.	15th April, 1932.	19th April, 1932.	22nd April, 1932.	27th April, 1932.	3rd May, 1932.	9th May, 1932.
Haemoglobin, gm. per 100 c.c.	L	13·31	13 31	13·6×	13 31	14 2×	15 42	16 87	17 84	17 51	17 18	17·51
Sugar, mg. % (Glucose).	L	38 17	43 48	39 84	34 01	39 37	43 4×	—	33 67	40·90	44 05	40·82
	U	27·18	42 37	26 74	29 43	33·56	36 36	—	30·77	32·26	33·56	30·21
Total N., gm. N %		2 919	2 884	2 940	2 968	3 136	3 087	3 178	3 234	3 332	3 290	3·143
Non-Protein Nitrogen, mgm. N %	L	29 34	22 72	3× 82	29 70	2× 42	21 42	30 7×	23 00	23 24	28 56	19 23
	U	9 68	13 04	25 00	21 72	16 94	13 63	19 73	15 00	18 29	18 87	12 40
Coagulable Nitrogen, gm. N %	L	2·899	2·461	2 907	2 938	3 107	3 066	3 147	3 209	3 309	3·261	3 124
	U	2 909	2·871	2 915	2 940	3 119	3·073	3 158	2 219	3 314	3 271	3 131
Urea, mg. N %	L	3·92	11 20	15 40	12 61	10 24	4 95	10 70	9 95	4 71	8·95	4·37
mg. U %	L	8 20	23 52	32 34	26 46	21 50	10·30	22 47	20 80	9 87	18·80	11·20
mg. N %	U	3 54	10 24	16 62	12 32	9 93	4·92	9 63	9 93	7 33	8·90	5 06
mg. U %	U	7 40	21 30	34 ×6	25 83	20 80	10 30	20 20	20 80	15 40	18·70	10·50
"Total" Creatinine, mg. N %	L	2 05	1 ×6	2 ×0	2 ×8	2 14	2 23	2 05	1·90	2·35	2·28	2 01
mg. TC %	L	5 54	5 02	7 58	7 78	5 76	6 00	5 54	5 14	6 36	6·61	5·40
mg. N %	U	1·44	1 44	2 05	2 36	1 56	1·90	1 40	1 40	1 60	1·75	1·34
mg. TC %	U	3 ×6	3 86	5 54	6 40	4 22	5·14	3·78	3 78	4·32	4·70	3·60
Uric acid, mg. N %	L	·33	27	·44	·49	·30	·29	21	·23	33	·29	27
mg. UA %	L	1 0	80	1 33	1 18	·89	·86	63	·69	1 00	·88	·80
mg. N %	U	—	—	20	13	13	13	—	—	—	·14	·12
mg. UA %	U	—	—	·61	40	—	38	—	—	—	41	·36
Amino-acid, mg. N %.	L	5 83	5 60	6 36	6 60	7 00	7 00	7 37	6 86	6·67	7·37	6·36
	U	4 83	3 94	5 18	5 43	4 12	4 67	5 38	4 52	4 83	4 00	4·12
Rest Nitrogen, mg. N %.	L	8 21	3 79	8 32	7 22	9 74	6·95	10 45	6·56	9·18	9·07	5·22
	U	13	5 24	95	1·48	1 33	2·01	3 32	·85	4·53	4 08	1·77

History : Bluetongue, June, 1929.
Weights : 1: 12·31 — ×6 lb.
22. 1.32 — 100 lb.
23. 2.32 — 93 lb.
29. 4.32 — 95 lb.
18. 5.32 — transferred to trypanosome experiment

TABLE 4.—Sheep 24158.

Date.	30th Jan. 1933.	24th Jan. 1933.	22nd Dec. 1932.	15th Dec. 1932.	30th Sept. 1932.	12th July 1932.	29th June 1932.	28th June 1932.	9th June 1932.	19th May 1932.	12th May 1932.	30th March 1932.	21st March 1932.	17th March 1932.	18th Dec. 1931.	17th Dec. 1931.	10th Nov. 1931.	5th Nov. 1931.
Haemoglobin, gm. per 100 c.c.	15·71	17·51	14·08	18·20	12·13	14·50	14·50	15·19	13·68	14·72	14·28	15·19	16·87	14·28	20·80	14·72	16·87	16·87
Sugar, mg. % (Glucose). — L	40·65	41·66	42·55	51·81	43·86	51·02	36·23	52·08	55·25	50·00	59·52	50·76	51·81	54·98	46·51	37·31	43·86	41·84
Sugar, mg. % (Glucose). — U	26·46	37·88	34·48	45·25	35·59	37·88	33·78	43·10	44·25	40·98	42·19	41·86	43·48	46·08	34·48	30·77	35·41	35·59
Total-N, gm. N %.	2·828	3·150	2·751	2·793	2·800	2·954	2·996	3·010	2·835	3·006	2·856	2·954	2·996	2·940	3·080	3·045	3·150	3·146
Non-protein N., mg. N % — L	27·78	31·74	36·36	48·38	20·83	19·73	26·54	25·46	—	20·00	22·22	19·61	27·78	31·42	20·00	35·70	25·52	23·42
Non-protein N., mg. N % — U	16·86	18·75	28·04	38·72	12·82	12·00	14·08	14·28	—	12·75	12·77	13·70	15·15	18·00	14·28	29·36	14·20	17·24
Coaguable Nitrogen, gm. N % — L	2·800	3·118	2·751	2·745	2·779	2·934	2·971	2·985	—	3·046	2·834	2·934	2·968	2·909	3·060	3·009	3·124	3·113
Coaguable Nitrogen, gm. N % — U	2·811	3·131	2·723	2·755	2·787	2·942	2·982	2·996	—	3·053	2·843	2·940	2·981	2·922	3·066	3·016	3·138	3·119
Urea. mg. N %	8·10	8·30	20·05	18·05	4·80	5·15	3·88	5·25	—	3·00	4·71	4·35	4·81	11·65	7·26	12·15	6·50	10·24
Urea. mg. U %	17·01	17·43	42·21	38·00	10·10	10·80	8·10	11·00	—	6·30	9·9	9·10	10·10	24·45	15·20	25·5	13·65	21·5
"Total" Creatinine. mg. N %	2·36	2·11	2·49	2·29	2·17	2·35	2·49	2·66	1·91	1·64	2·11	2·14	2·17	2·01	—	—	—	2·14
"Total" Creatinine. mg. TC %	6·36	5·68	6·47	6·16	5·84	6·36	6·74	7·2	5·14	4·40	5·68	5·76	5·84	5·40	—	—	—	5·76
Uric acid. mg. N % — L	·38	·49	·43	·21	·27	·27	—	·31	·27	·30	·29	·29	·27	·41	·42	·38	·32	·48
Uric acid. mg. UA % — L	1·14	1·46	1·28	·64	·82	·82	—	·94	·82	·91	·86	·86	·81	1·24	1·25	1·14	·97	1·43
Uric acid. mg. N % — U	·11	·20	·15	—	·08	·12	—	·16	·17	·15	·10	·13	·15	·16	·13	—	—	—
Uric acid. mg. UA % — U	·83	·60	·45	—	·24	·36	—	·47	·50	·44	·31	·39	·44	·47	·38	—	—	—
Amino-acid, mg. N % — L	5·94	7·61	7·00	5·00	6·86	5·18	5·83	5·60	5·22	5·49	6·06	7·78	5·24	6·10	7·00	6·03	7·14	6·83
Amino-acid, mg. N % — U	4·60	4·73	5·93	4·35	3·70	3·47	4·09	3·68	3·13	3·50	4·09	5·83	3·60	4·00	4·00	4·12	6·07	5·00
Rest Nitrogen, mg. N % — L	11·00	11·26	5·39	26·55	6·73	6·78	13·84	11·64	3·22	9·57	5·05	5·05	12·27	11·25	11·02*	16·08*	12·67*	3·73
Rest Nitrogen, mg. N % — U	1·84	3·77	1·01	15·82	2·86	3·74	7·07†	5·01	3·13	4·57	1·99	3·68	3·10	1·04	2·89*	8·31*	4·41*	0·43

* Includes "Total"-creatinine-N.
† Includes Urea N.

History : Bluetongue, June, 1929.
Weights :
18.12.31 — 102 lb.
22.1.32 — 118 lb.
19.2.32 — lambed, lamb No. 33597.
22.2.32 — 95 lb.
29.4.32 — 98 lb.
26.5.32 — 95 lb.
22.7.32 — 107 lb.
24.8.32 — 115 lb.
11.10.32 — lambed.
29.12.32 — 95 lb.

TABLE 5.—Sheep 24160.

Date.		5th Nov., 1931.	10th Nov. 1931.	17th Dec. 1931.	18th Dec. 1931.	17th March. 1932.	21st March, 1932.	30th March, 1932.	18th May, 1932.	17th May, 1932.
Haemoglobin, gm., per 100 c.c.	L	11 99	15 42	13 31	14 25	15 98	15 71	16 87	18 84	17 94
Sugar, mg.%	L	41 84	34 48	42 00	41 00	44·25	43 67	45 45	59·52	50 00
(glucose)	U	34 48	32 30	34 70	35 70	34 25	31 65	32 36	41·84	38·76
Total-N., gm. N %		3 150	3 017	2 709	2 772	3 220	3 136	3 241	3·220	3·234
Non-Protein N., mg.%.	L	20 34	—	25 20	20 09	36 82	28 04	25 00	21·58	24·46
	U	13 76	14 63	16 66	13 63	22·22	16 76	11 45	14·85	12·82
Coaguable Nitrogen, gm. N %.	L	3·130	3 002	2 684	2 752	3 183	3 108	3 216	3·198	3·210
	U	3 136	—	2 692	2 758	3 198	3 119	3·230	3·205	3·221
Urea. mg. N %	L	4·55	5·61	5 66	6 66	16 39	6 69	6 00	4·18	4·88
mg. U %	L	9·50	11·80	11 88	13 98	34 40	14 00	12 60	8·77	10·20
mg. N %	U	5·52	5 07	6 30	7 33	13 29	7·33	5 17	4·18	5·12
mg. U %	U	11 60	10 60	13 20	15 40	27 80	15 40	10 80	8·70	10·80
"Total"-Creatinine. mg. N %	L	2 14	—	—	—	1·90	2 01	2·14	2·23	1·90
mg. TC %	L	5 76	—	—	—	5 14	5 40	5·76	6·00	5·14
mg. N %	U	1·97	—	—	—	1·41	1·41	1·67	1·49	1·86
mg. TC %	U	5·32	—	—	—	3 80	3·80	4·50	4·00	5·02
Uric acid. mg. N %	L	·46	·31	·40	·45	·44	·27	·31	·30	·24
mg. UA %	L	1 39	·94	1 21	1·36	1 31	·80	·94	·91	·73
mg. N %	U	—	—	·14	·11	·17	·12	·13	—	·12
mg. UA %	U	—	—	·43	·34	·52	·35	·40	—	·36
Amino-acid, mg. N %.	L	6 83	6 09	6 73	7 00	7 00	7·78	7·78	7 00	7 00
	U	6 09	4 12	4 54	4 12	4 12	4 18	5 18	8·09	4·16
Rest Nitrogen, mg. N %.	L	6 36	—	12 41*	5·89*	11 09	11·29	8 77	7·87	10 44
	U	·18	5 44*	5 68*	3·07*	3 23	3 72	— 70	3 09	1·55

* Includes "Total"-Creatinine-N.

History: Bluetongue, June, 1929.
Weights: 18.12.31 — 85 lb.
21.1.32 — 83½ lb.
23.2.32 — 81 lb.
29.4.32 — 85 lb.
18. 5.32 — transferred to trypanosome experiment.

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TABLE 6.—Sheep 24163.

Date.		6th Nov., 1931.	10th Nov., 1931.	12th Dec., 1931.	18th Dec., 1931.	16th March, 1932.	21st March, 1932.	31st March, 1932.	13th May, 1932.	19th May, 1932.	27th May, 1932.	3rd June, 1932.	7th June, 1932.	5th July, 1932.	7th July, 1932.
Haemoglobin, gm. per 100 c.c.		10 35	14·28	14 08	13·68	15·19	14·72	13 31	14 95	16 87	11·67	13 87	16·29	15·42	15·42
Sugar mg. % (Glucose).	L	42·19	34·48	45 45	41·32	38 31	47·74	42 37	37·59	38·31	46·73	41·84	45·05	45 05	47·62
	U	40 0	33 83	34·48	35·71	30·77	37·59	37·74	29·07	31 35	39 53	39·22	36·36	35 21	37·45
Total N., gm. N %	L	2·380	2·926	2 590	2·716	3·248	2·912	2·758	2·947	3 164	2 576	2 870	3 003	3 052	3·066
Non-protein Nitrogen, mg. N %	L	13·39	20·40	28 04	35 70	27·26	29·42	16·66	15 00	17 54	14 08	15 68	16 94	19·48	15·39
	U	8·72	13·12	20·70	27·26	16 76	17·65	12 50	11 68	10 00	9 68	11 95	10 57	11 53	10·91
Coagula Ne Nitrogen, gm. N %.	L	2·367	2·920	2·562	2·680	3 221	2·883	2 741	2 932	3·146	2 562	2·853	2·986	3 033	3·051
	U	2·371	2 913	2·569	2·689	3 231	2 894	2 745	2·935	3 154	2·566	2·858	2 992	3·040	3·055
Urea. mg. N %, mg. U %	L	3·90 / 8 19	4·85 / 9·13	9·52 / 19·95	15 66 / 32 8	9 31 / 19 53	6 35 / 13·8	4 72 / 9 9	3·26 / 6·8	3 16 / 6 6	2 70 / 5 67	2·84 / 5·9	1·85 / 3·8	3·76 / 7·8	2·57 / 5·30
mg. N %, mg. U %	U	3·46 / 7·21	3·42 / 7 2	9 52 / 19·9	15·66 / 32 90	9·42 / 19·74	6·32 / 13 23	5 25 / 11 00	4 4 / 8 0	2 77 / 5 7	2 31 / 5 25	2 84 / 5 9	1 25 / 2 6	2 71 / 3·6	t. 1 / t. 1
"Total" creatinine. mg. N %, mg. TC %	L	1·71 / 4·64	— / —	— / —	— / —	1·86 / 5·02	1 90 / 5 14	1 97 / 5 32	1·82 / 4·90	1·60 / 4 32	1·82 / 4 90	2 01 / 5 40	2 09 / 5 68	1·82 / 4 90	1 86 / 5·02
mg. N %, mg. TC %	U	1·49 / 4 0	— / —	— / —	— / —	1·49 / 4 0	1 34 / 3 36	1·78 / 4·80	1·14 / 3 08	1 38 / 3 72	1 56 / 4 16	1·45 / 3·86	1·45 / 3 86	1·60 / 4 32	1·52 / 4·08
Uric Acid. mg. N %, mg. UA %	L	·18 / ·53	·27 / ·80	·25 / 76	33 / 1 0	26 / 78	20 / ·60	·23 / ·68	·22 / ·67	·25 / ·76	·18 / ·53	18 / ·53	·23 / ·68	·28 / ·84	·27 / ·80
mg. N %, mg. UA %	U	— / —	— / —	— / —	— / —	17 / ·51	·12 / ·35	13 / 39	— / —	·13 / ·40	·08 / ·25	·08 / 24	·11 / 32	·19 / 57	·14 / ·43
Amino-acid, mg. N %.	L	5 15	5 28	6 86	6 36	5 83	5 47	6·67	5 60	5 18	5 60	5 83	5 58	4 06	4 97
	U	4 67	3 89	4·83	4 83	4 38	4 0	4 83	3·59	3 20	4·52	4 67	4 12	3·18	3 89
Rest Nitrogen, mg. N %.	L	2 45	10·50*	11 41*	13 34*	10 0	15·50	3 07	4 10	7 35	2 78	5 72	7 19	9 56	5 72
	U	90	5 81*	6 35*	6 77*	1 30	5 87	51	2 95	2 46	1 01	2 92	3 64	3·85	5·36

* Includes "Total" creatinine N.

History: Bluetongue, June, 1929.
Weights: 18.12.31 — 77 ℔.
21. 1.32 — 88 ℔.
23. 2.32 — 85 ℔.
29. 4.32 — 87 ℔.
26. 5.32 — 85 ℔.
22. 7.32 — 92 ℔.
24. 8.32 — 92 ℔.
24. 8.32 — 94 ℔.
15. 9.32 — died.

EXPLANATION OF GRAPHS I AND II.

In order to emphasize the percentage variations of the various constituents (except haemoglobin, total nitrogen and sugar) both in the "laked" and "unlaked" blood filtrates over the whole period, nitrogen partition curves have been drawn, incorporating the monthly averages of all the different experimental data collected of a group as points for plotting the curves (Tables 7 to 17, 21 to 31).

Such nitrogen partition curves have only been drawn of groups A and B. As the curves of the other groups were found to show the same general tendencies, they were omitted.

GRAPH I.

Percentage Curves. Nitrogen Partition of Non-Protein Nitrogen.

Group A.
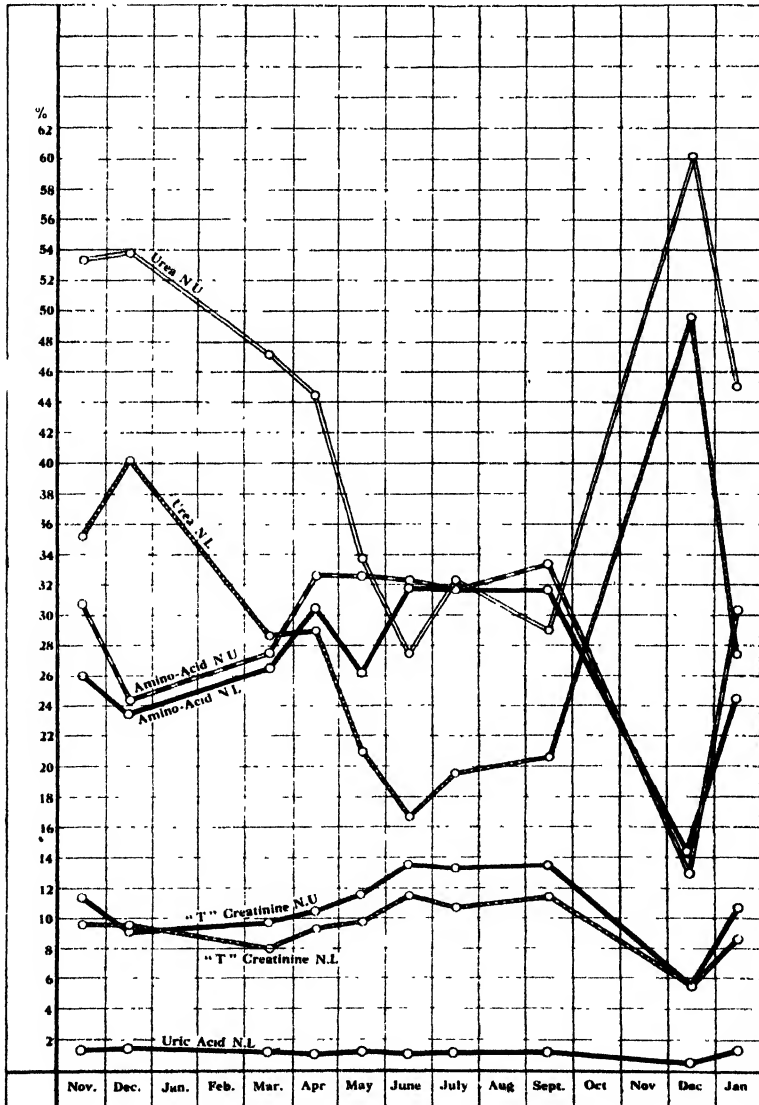
Group A (adult ewes.)

TABLE 7.—*Haemoglobin (Hb.)* gm. per 100 c.c.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	15·6	14·9	—	—	13·9	13·4	—	—	17 3	—	13 3	--	—	14·6	13·8
24136	12·3	16·1	—	—	12 9	14·0	13·7	13·6	—	—	10 9	—	—	15·4	12·5
24156	13·3	13·5	—	—	—	16·4	17·3	—	—	—	—	—	—	—	—
24158	16·9	17·7	—	—	15·5	—	14·5	14·5	14·5	—	12 1	—	—	16 1	16 6
24160	13·7	13·8	—	—	16·2	—	18·3	—	—	—	—	—	—	—	—
24163	12·3	13 9	—	—	14·4	—	14·5	15 1	15 4	—	—	—	—	—	—
Av...	14·0	14·9	—	—	14 8	14 8	15·6	14·3	16·0	—	12 1	—	—	15 4	14·3

It is evident from the average haemoglobin figures per month of all the sheep, as well as from the monthly averages of individual sheep that there is no definite indication of a steady decrease or steady increase during the period. Plotted as a graph it would appear as a zigzag line. It may, however, be concluded that no seasonal variation in the haemoglobin content of blood takes place.

Neser (1923), working with horse blood, drew attention to similar variations in his researches on the "Percentage volume or count of red cells" in one and the same horse examined over short periods. He referred more particularly to variations encountered in the same animal, at the same time, in different parts of the circulation (venous and capillary), but his results obviously also apply to differences found in the same animal from day to day (in the absence of pathological conditions). (*Vide* also Chemical Blood Ssudies IV.)

Attention is drawn to the fact that Hb. figures at the beginning of the experiment were abnormally high or low. (See Tables 1, 5 and 6.)

The Hb. figures vary from 10·35 to 20·60 gm. per 100 c.c., the average of all the figures over the whole period of 15 months, being 14·7 gm. per 100 c.c.

The following table illustrates the distribution more clearly by showing the number of analyses falling into each particular group:—

From 10–11 gm. per 100 c.c. 2
11–12 gm. per 100 c.c. 3
12–13 gm. per 100 c.c. 10
13–14 gm. per 100 c.c. 15
14–15 gm. per 100 c.c. 22
15–16 gm. per 100 c.c. 12
16–17 gm. per 100 c.c. 9
17–18 gm. per 100 c.c. 9
18–19 gm. per 100 c.c. 2
19–20 gm. per 100 c.c. 0
20–21 gm. per 100 c.c. 1

39 per cent. of the determinations lie between 14 and 15 and 58 per cent. between 13 and 16 gm. per cent.

TABLE 8.—*Sugar (" Laked ") mg. per cent.*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	48·4	45 0	-	—	48·5	45·5	—	—	43 2	—	38·6	—	—	49·1	44·9
24136	31·1	31 7	—	—	41·1	40·3	34·1	47 0	—	—	43·3	—	—	42·0	34·9
24156	40 8	36·9	--	—	—	39·4	42·4	—	—	—	—	—	—	—	—
24158	42·9	41·9	-	—	52·5	—	54·8	47 9	51 0	—	43 9	—	—	47 2	41 2
24160	38·2	41·5	-	···	44·5	—	54 8	—	—	—	—	—	—	—	—
24163	38 3	43 4	—	--	42·8	—	40·9	43 4	46 3	—	—	—	—	—	—
Av...	39 9	40·1	—	—	45·8	41 5	44 4	46·4	46 0	—	41·9	—	—	46 9	40 4

TABLE 9.—*Sugar (" Unlaked ") mg. per cent.*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar	Apl.	May.	June.	July.	Aug	Sept.	Oct.	Nov.	Dec.	Jan.
15398	36·8	35 4	—	--	40 3	35 7	—	—	34 1	—	29 4	—	—	36 5	34·7
24136	26 9	22 3	—	—	35 0	33·7	30 8	40 3	—	—	40 0	—	—	35 5	31·4
24156	34 8	28 0	—	—	—	33 2	32 0	—	—	—	—	—	—	—	—
24158	35 5	32 6	-	--	43 7	-·-	41·6	40 4	37 9	—	35·6	—	—	39 9	32·2
24160	33 4	35 2	—	-	32·8	—	40 3	—	—	—	—	—	—	—	—
24163	36 7	35 1	--	-	35 4	-·-	33 3	37·8	36 3	—	—	—	—	--	—
Av...	33·9	31·4	--	—	37 1	34 1	35·4	39 7	35·7	—	35 0	—	- -	37·5	32·6

" Laked " filtrates.
min.-max. variation
27–59·5 mg. per cent.
av. 43·3 mg. per cent.
av. diff. 8·2 mg. per cent.

" Unlaked " filtrates.
min-max. variation
19·6–51·3 mg. per cent.
av. 35·1 mg. per cent.

The following table indicates the distribution:—

" Laked " filtrates.

mg. %	Occurrence.
25–30	1
30–35	9
35–40	14
40–45	31
45–50	14
50–55	9
55–60	3

" Unlaked " filtrates.

mg. %	Occurrence.
15·–20	1
20–25	0
25–30	10
30–35	34
35–40	23
40–45	12
45–50	2
50–55	1

38 per cent. of the " laked " filtrate determinations lie between 40 and 45 mg. per cent., and 73 per cent. between 35 and 50 mg. per cent., while 41 per cent. of the " unlaked " filtrate determinations lie between 30 and 35 and 83 per cent. between 30 and 45 mg. per cent.

Comparison.

The percentage difference of the " laked " and " unlaked " figures varies from 2·3 to 28·8 per cent., with the average difference of 8·2 mg. per cent. (43·3–35·1), i.e. the average difference is 19 per cent.

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The " laked " blood sugar figures are always more than that of the " unlaked " filtrates. By comparison of the two curves (graph III) it is clear that the percentage difference decrease gradually towards winter (except from March to May), because the sugar content increases and the difference in mg. per cent. remains about the same (see Tables 8 and 9). Note the course of the curves from September, 1932, to the end.

The averages of sheep 24136 are exceptionally low and of S.24158 exceptionally high.

Total Nitrogen (T.N.). gm. per cent.

The total nitrogen varies from 2·7 to 3·3 gm. N. per cent. with an average of 3·0 gm. N. per cent.

By comparing the different figures of each set of figures of each individual sheep, it is noticed that the T.N. remains fairly constant.

The relation between the haemoglobin and total nitrogen is affirmed because usually the figures increase or decrease simultaneously, e.g. on Table 1 (4.7.32) both Hb and T.N. are high and on Table 2 (29.6.32) both are low. For individual differences compare Tables 2 and 3, those of 2 being low (e.g. 2·72, 2·66, 2·70, 2·84 etc.), and of 4 high (e.g. 3·14, 3·15, 3·05, 3·08, etc.).

TABLE 10.—*Non-Protein Nitrogen mg. per cent. " Laked ".*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	22·5	30·0	—	—	27 8	20 9	—	—	20 5	—	19 2	·	—	44 0	24 5
24136	25·2	25·9	—	—	18 3	17·4	15 6	18 5	—	—	17 1	—	—	37 5	21 8
24156	26·5	31·5	—	—	- -	25 9	42 4	—	—	—	—	—	—	—	- -
24158	24·5	27 9	—	—	26 3	—	21 1	25 5	19 7	---	20·8	—	--	42 4	29 8
24160	20·3	22·6	—	—	30 0	—	23 0	—	—	—	—	—	—	—	—
24163	16·9	31·9	—	—	24 5	—	15 5	16 8	17 4	· ·	—	—	—	—	—
Av...	22 0	28·3	—	—	25·5	22 2	19 5	20 3	19 2	—	19 1	·	—	42 0	25·3

TABLE 11.—*Non-protein nitrogen mg. per cent. " Unlaked ".*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept	Oct.	Nov.	Dec.	Jan.
15398	15 2	24·3	—	—	17 1	12 9	—	—	11 6	—	15 0	—	—	36·7	15·4
24136	19·8	18·1	—	—	12 4·	13 7	11·4	10·9	—	—	12·5	—	-	31 6	16 0
24156	11·4	23·4	—	—	—	16·7	15 6	—	—	—	—	—	—	—	—
24158	15·7	21·8	—	--	15 6	—	12·8	14 4	12 1	—	12 8	--	·	33 4	15 3
24160	14·2	15·1	—	—	16·8	—	13·8	—	—	—	—	—	—	—	—
24163	10 9	23·9	—	—	15 6	—	10 5	11 3	11 2	—	—	—	—	—	—
Av...	14 5	21·1	—	—	15 5	14·7	12·6	12 1	11 5	—	13 4	·	—	34·3	15·6

" *Laked* " *filtrates.*
Minimum-maximum variation, 13·39–48·38, mg. % N.
Average, 24·0, mg. % N.
"*Unlaked*" *filtrates.*
Minimum-maximum variation, 8·72–39·46, mg. % N.
Average, 16·2, mg. % N.
Average difference, 7·8, mg. % N.

The following Table indicates the distribution:—

" Laked " filtrates.		" Unlaked " filtrates.	
mg. % N	Occurrence.	mg. % N	Occurrence.
Below 10	3	Below 10	4
15–20	23	10–15	43
20–25	29	15–20	21
25–30	18	Above 20	15
Above 30	13		

30 per cent. of the " Laked " filtrate determinations lie between 20–25 mg. per cent. N.

81 per cent. of the " Laked " filtrate determinations lie between 15–30 mg. per cent. N.

52 per cent. of the " Unlaked " filtrate determinations lie between 10–15 mg. per cent. N.

77 per cent. of the " Unlaked " filtrate determinations lie between 10–20 mg. per cent. N.

Comparison.

The difference of the " laked " and " unlaked " figures varies from 11·5 to 54·0 % with an average of 34·3 % and an average difference of 7·8 in mg. % N (24·0–16·2).

It is clear from Graph IV. that the percentage difference of N.P.N. in the plasma and cells, increased towards the winter, because the graphs are always about parallel when descending. This difference in mg. % N remains about the same (December, 1931, 25 %; July, 1932, 39 %). The N.P.N. increases from November to December and reaches the minimum towards July. It changes not much towards September, but towards December (1932), when green fodder was again included in the ration the Graph reaches the maximum, while in January when the green fodder was again excluded it declined again. Undoubtedly this change can partly, if not wholly, be ascribed to the change in the ration from December (1932) to the end of the experiment. But that the seasonal factor may play a part, cannot at this stage of the experiment be definitely excluded, and this aspect is still under investigation.

The individual differences are noticeable, e.g. S. 24156 which is high, and S. 24163 which is low.

TABLE 12.—Urea Nitrogen mg. % " Laked ".

Sheep No.	Nov.	Dec.	Jan	Feb.	Mar.	Apl.	May	June.	July.	Aug	Sept	Oct.	Nov.	Dec.	Jan
15398	10·8	15 2	—	—	5 7	4 6	—	—	3·6	—	3 4	—	—	25 0	5·7
24136	10 7	10·7	—	—	5 9	6 5	3·3	3 1	—	—	3·6	—	—	15 9	6·9
24156	7·6	14 0	—	—	—	8·1	7·2	—	—	—	—	—	—	—	—
24158	8·4	9 7	—	—	6·9	—	3 9	4 6	5 2	—	4 8	—	—	19 0	8 2
24160	5 1	6·2	—	—	9·7	—	4·5	—	—	—	—	—	—	—	—
24163	4·1	12·6	—	—	6·8	—	3 0	2 3	3 2	—	—	—	—	—	—
Av...	7·8	11·4	—	—	7 3	6 5	4·3	3 3	3 7	—	3 9	—	—	20·8	6 9

The urea nitrogen varies from 1·85 to 31·5 mg. % N, with an average of all the figures over the 15 months of 7·56 mg. % N.

The following Table indicates the distribution:—

mg. % N	Occurrence.
1–2	1
2–3	4
3–4	14
4–5	16
5–6	8
6–7	8
7–8	3
8–9	3
above 9	15

42 % of the determinations lie between 3 and 5 mg. % N.

A table of the " unlaked " figures will differ very little from Table 12 because the amount of urea in the cellular and plasma fraction of the blood is practically the same. Consequently only one curve for the " laked " and " unlaked " figures on Graph IV has been plotted. It must be stated, however, that with a few exceptions the " laked " figures are usually slightly more than the " unlaked ". The symbols " t.l." (too low) indicate that the urea content was too low for accurate colorimetric readings to be taken, e.g. vide Table 1 (8.7.32) and Table 6 (7.7.32).

The maximum figure obtained for the " unlaked " filtrates is 31·53 mg. % N.

Comparison.

The urea nitrogen curve on Graph IV approximately parallels the N.P.N. curves of Group A. On account of the fact that the urea nitrogen curves practically coincide, it is evident that the urea nitrogen is approximately equally divided between the blood cells and plasma.

Although the urea N and N.P.N. curves are practically parallel, the urea N curve obviously is much lower, and from this it is apparent that the urea nitrogen percentage of the N.P.N. drops considerably from December (1931) to June (see Graph I and Table 12). Concerning the change in Graph IV and consequently also on Graph I the same factor that is mentioned (diet) under the N.P.N. is applicable here.

On Graph I the urea N percentage curves of the " laked " and " unlaked " blood filtrates are plotted. Although the urea N (mg. %) of blood determined on the " laked " and " unlaked " blood filtrates are the same, the urea N calculated as percentage of the respective N.P.N.'s (in mg. %) for the two filtrates, differ, because the N.P.N. for the two filtrates differ (Table 10 and 11). The " unlaked " urea N percentage curve runs much higher than the " laked " urea N percentage curve, since the respective N.P.N. (mg. %) is much lower. Note the individual differences (S. 15398 and S. 24163, see Table 12).

TABLE 13.—" Total " Creatinine N mg. % (" Laked ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	1·9	2·6	—	—	2 2	2 0	—	—	2 1	—	2·0	—	—	2 4	2·4
24136	2·5	2·6	—	—	2 1	1 9	1·8	2 5	—	—	2·3	—	—	2·0	1·8
24156	1·9	2 8	—	—	—	2 1	2·1	—	—	—	—	—	—	—	—
24158	2·1	—	—	—	2·1	—	1·9	2·4	2 35	—	2 2	—	—	2·4	2·2
24160	2 1	—	—	—	2·0	—	2 1	—	—	—	—	—	—	—	—
24163	1·7	—	—	—	1 9	—	1 7	2 1	2 1	—	—	—	—	—	—
Av...	2·1	2·7	—	—	2 04	2 0	1 9	2 3	2·0	—	2 2	—	—	2·3	2·1

TABLE 14.—" Total " Creatinine N mg. % (" Unlaked ").

Sheep No	Nov.	Dec.	Jan	Feb.	Mar.	Apl.	May.	June	July	Aug.	Sept.	Oct	Nov.	Dec.	Jan.
15398	1·5	2·1	—	—	1·8	1 5	—	—	1·4	—	1·7	—	—	1·9	1·2
24136	1 9	2 2	—	—	1 5	1 4	1 2	1 7	—	—	2 0	—	—	1 9	1·5
24156	1 4	2 2	—	—	—	1 6	1 5	—	—	—	—	—	—	—	—
24158	1 8	—	—	—	1·5	—	1·5	1 8	1·7	—	1 7	—	—	1 9	1·7
24160	1·9	—	—	—	1 5	—	1 7	—	—	—	—	—	—	—	—
24163	1·5	—	—	—	1 5	—	1 4	1 5	1·6	—	—	—	—	—	—
Av ..	1 6	1 9	—	—	1 6	1·5	1 5	1 7	1 6	—	1 8	—	—	1·9	1·7

" Laked Filtrates.

Minimum-maximum variation, 1·49–2·88 mg. N %.
Average, 2·14 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, ·95–2·08 mg. N %.
Average, 1·62 mg. N %.
Average difference, 0·52 m. N% (laked to unlaked).

Comparison.

The difference between " laked " and " unlaked " " total " creatinine nitrogen lies between 2·1 and 48·9 % and the average difference is 24·5 %.

It is evident from the curves in mg. % N (Graph IV) that the percentage, difference between " laked " and " unlaked " figures increases towards winter since the curves run approximately parallel but descending (Nov. 21 %, Jul. 25 %).

On Graph I the percentage curve rises because, although the average on Tables 13 and 14 (Graph IV) fall a little bit towards winter, they do not fall proportionally as much as the respective non-protein nitrogens (see Tables 10 and 11).

Note the individual differences, e.g. on Table 13, c.f. S. 24163, which is low, with the others.

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TABLE 15.—*Uric-acid Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	0·34	0·41	—	—	0·45	0 27	—	—	0 35	—	0 27	—	--	0 28	0·37
24136	0·18	0·19	—	—	0·20	0·11	0 28	—	—	—	0 22	--	—	t.l.	0·18
24156	0·30	0·41	—	—	—	0·27	0 28	0·13	—	—	—	—	—	—	—
24158	0·40	0·40	—	—	0·32	0·29	0·29	—	0·27	—	0·27	—	—	0 32	0·44
24160	0·38	0·42	—	—	0·34	0·27	—	—	—	—	—	—	—	—	—
24163	0·22	0·29	—	—	0 23	0·23	0 22	0 20	0 27	—	—	—	—	—	—
Av...	0·29	0·36	—	—	0·29	0·23	0·26	0 23	0 25	—	0 25	—	—	0 24	0·33

Minimum-maximum variation, from less than ·10 to ·48 mg. % N.

Average, ·28 mg. % N.

" *Unlaked* ".—No table of this is given, because too many of the filtrates contained undeterminable small quantities uric acid N. An average will thus be of no value.

Maximum, ·30 mg. % N.

Comparison.

The uric acid N (mg.) in " laked and " unlaked " blood filtrates is very variable (e.g. *vide* Table 4) and so is also the relation in the two filtrates, even in the individual cases (e.g. Table 4, 5.11.31 and 18.12.31). In the first case the " unlaked " uric acid N was undeterminably low and the " laked " figure (·48 mg. % N) is the highest level ever obtained in these analyses. On the other hand, the " laked " figures of S. 24136 were always extremely low (Table 15). The curve (Graph IV) rises a bit towards December (1931), but maintains its level from March to July (c.f. averages on Table 15). The " unlaked " uric acid nitrogen curve is not drawn for reasons already given.

The percentage curve is approximately level at 1·3 % N (Graph I). Note the individual low averages of S. 24136 (·18, ·19, ·20, ·11, etc.), and the higher averages of S. 15398 (·34, ·41, ·45, ·27, etc.) (*vide* Table 15).

TABLE 16.—*Amino-Acid Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	5·4	7 1	—	—	7 5	5 6	—	—	5·2	—	6 7	—	—	6 5	6·6
24136	4 7	5·7	—	—	5 6	5 8	4 9	4 7	—	—	4 7	—	—	5·1	5·2
24156	5·7	6·5	—	—	—	6 9	6·9	—	—	—	—	—	—	—	—
24158	6·4	7·1	—	—	7 4	—	5·8	5 6	5 2	—	6 9	—	—	6·0	6·8
24160	6·5	6·9	—	—	7 5	—	7·0	—	—	—	—	—	—	—	—
24163	5·2	6·6	—	—	6 0	—	5 5	5·7	7·6	—	—	—	—	—	—
Av...	5·6	6·6	—	—	6 8	6·8	5·9	5·3	6·2	—	6·1	—	—	6 0	6·2

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TABLE 17.—*Amino-Acid Nitrogen mg. % ("Unlaked").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
15398	4·4	6 2	—	—	5 2	4 8	—	—	3 9	—	5 4	—	—	4 2	4·7
24136	4·3	4 5	—	—	4·7	4·9	3 9	3 9	—	—	4 4	—	—	3 7	4·8
24156	4·4	5·2	—	—	—	4·7	4 1	—	—	—	—	—	—	—	—
24158	4·4	4·8	—	—	5 1	—	3 8	3 6	3·5	—	3 7	—	—	4·9	4·7
24160	5·1	4 3	—	—	4 5	—	5·1	—	—	—	—	—	—	—	—
24163	4·3	4 8	—	—	4 4	—	3·8	4·4	3 5	—	—	—	—	—	—
Av...	4·5	5 1	—	—	4 3	4 8	4 1	3 8	3·7		4·5	—	—	4·5	4·7

"*Laked*" *Filtrates.*

Minimum-maximum variation, 3·68–8·75 mg. % N.
Average, 6·37 mg. % N.

"*Unlaked*" *Filtrates.*

Minimum-maximum variation, 3·13–6·67 mg. % N.
Average, 4·43 mg. % N.
Average difference, 1·94 mg. % N.

Comparison.

The percentage difference of "laked" and "unlaked" figures
varies from 9·3 to 46·7, with an average of 30·5. The curves in
mg. % N run fairly constant (Graph IV) towards April (1932) and
fall to a minimum in June (Tables 16 and 17). In November (1931)
the difference was just over 1 mg. % N, but gradually the difference
increases to 2 51 mg. % N in July, at the cost of the "unlaked"
figures, that is in the "laked" the content remained fairly constant,
whereas in the "unlaked" there was an earlier decrease. The per-
centage difference increased towards winter (December, 1931, 23 %,
July 40 %, see Graph IV).

On Graph I the percentage curves are approximately parallel,
because although the averages (see Tables 16 and 17) decrease some-
what, they do not do so as proportionally as the respective non-
protein nitrogens. These curves were influenced by the change in
the ration, rising with the withdrawal of green feed and falling with
the addition of it.

Note the individual differences, e.g. S. 15398 being fairly high
(5·4, 7·1, 7·5, 5 6, etc.), and S. 24136 being fairly low (4·7, 5·7,
5·6, 5·8, etc.).

(*b*) GROUP B, TABLES (18-31), GRAPH II AND DISCUSSION OF GROUP B.

Sheep No. 33208.

,, No. 33589.

,, No. 33597.

TABLE 18.—Sheep 33208.

Date		14th March, 1932.	16th March, 1932.	23rd March, 1932.	31st March, 1932.	14th April, 1932.	18th April, 1932.	21st April, 1932.	25th April, 1932.	28th April, 1932.	3rd June, 1932.	7th June, 1932.	11th July, 1932.	13th July, 1932.	29th Sept., 1932.	21st Dec., 1932.	25th Jan., 1933.	1st Feb., 1933.
Haemoglobin gm. per 100.....	U	16·54	14·08	15·42	15·42	12·13	14·28	13·68	13·12	13·31	11·96	14·49	13·68	13·87	11·96	14·95	16·54	12·59
Blood Sugar (glucose) mg. %....	L	52·36	55·55	52·08	45·05	52·91	48·31	52·36	52·63	60·98	55·55	67·11	44·84	51·55	59·17	49·75	42·74	48·54
	U	46·73	52·08	49·02	39·68	47·17	46·31	46·72	58·82	55·55	51·28	62·11	43·29	44·44	51·22	45·66	35·84	43·29
Total N. gm. N %....	U	3·066	3·024	3·048	3·024	2·702	2·842	2·730	2·604	2·646	2·835	2·611	2·926	2·800	2·632	3·024	3·129	2·590
Non-protein nitrogen mg. %	L	20·70	19·73	30·92	23·24	27·26	15·87	17·97	17·44	23·16	15·09	14·85	15·23	15·71	18·07	32·60	27·40	24·70
	U	12·82	12·35	23·24	14·71	24·58	11·53	11·49	13·83	15·00	9·73	11·41	10·49	10·71	11·36	25·86	17·65	18·41
Coagulable Nitrogen gm. N %....	L	3·045	3·004	3·031	3·001	2·675	2·826	2·712	2·587	2·623	2·819	2·596	2·911	2·785	2·614	2·991	3·102	2·565
	U	3·053	3·012	3·015	3·009	2·677	2·830	2·715	2·590	2·631	2·825	2·600	2·916	2·790	2·621	2·998	3·111	2·572
Urea mg. N %	L	—	7·73	16·55	6·40	10·90	3·16	3·31	5·85	6·09	2·57	2·57	4·26	t.l.	3·76	17·69	8·01	9·00
mg. U %	L	—	16·20	34·70	13·4	22·9	6·60	7·0	12·25	12·80	5·40	5·40	8·90	t.l.	7·00	37·17	16·80	18·90
mg. N %	U	6·47	6·27	16·94	6·55	10·90	2·82	3·20	4·40	6·24	2·54	2·33	3·26	t.l.	3·70	18·05	7·58	11·80
mg. U %	U	13·56	13·16	35·60	13·70	22·89	5·90	6·72	9·24	13·10	5·50	4·90	5·96	t.l.	7·90	38·01	15·96	24·78
"Total Creatinine" mg. N %	L	1·82	1·64	1·56	2·66	2·14	1·75	1·56	1·34	1·67	1·78	1·90	2·16	2·58	1·82	1·90	1·82	1·89
mg. TC %	L	4·90	4·40	4·22	7·2	5·76	4·80	4·22	3·6	4·50	4·80	5·14	5·84	6·96	4·90	5·14	4·90	5·14
mg. N %	U	1·38	1·34	1·67	1·56	2·05	1·44	1·34	1·26	1·34	1·55	1·51	1·75	2·04	1·45	1·60	1·26	1·56
mg. TC %	U	3·72	3·60	4·50	4·22	5·54	3·88	3·60	3·42	3·60	4·16	4·08	4·70	5·50	3·92	4·32	3·86	4·16
Uric Acid mg. N %	L	·23	·11	·15	·14	·16	—	—	·13	·18	·12	·19	·13	·15	·18	·23	·26	·20
mg. UA %	L	·68	·34	·45	·42	·49	—	—	·39	·55	·37	·57	·40	·46	·53	·69	·78	·59
mg. N %	U	—	—	·12	·12	·13	·11	—	·13	—	·07	·12	·08	·09	·09	—	·17	·10
mg. UA %	U	—	—	·36	·36	·39	·32	—	·38	—	·22	·36	·25	·28	·28	—	·50	·29
Amino Acid mg. N %....	L	6·5	5·35	5·83	7·14	5·47	7·00	6·36	6·36	5·83	5·53	4·67	4·81	4·67	5·15	6·36	5·83	6·03
	U	4·67	4·38	—	6·17	5·00	5·18	4·67	5·0	5·88	4·24	3·62	3·41	4·12	4·52	5·26	5·38	5·60
Rest Nitrogen mg. N % .	L	12·15*	4·57	6·83	6·90	8·39	3·93†	6·74†	3·76	9·39	5·79	5·52	3·87	8·31	6·96	5·42	11·48	7·58
	U	·30	·30	4·51‡	·31	6·50	1·98	2·28†	3·04	2·04	1·23	3·83	1·99	4·46	1·54	− 0·05	3·26	− 0·67

*Includes Urea N. † Includes Uric Acid N. ‡ Includes Amino Acid N.

History; Born at Onderstepoort 29.12.31 of Ewe 24136 (*vide* Group A).
Weight;
29. 4.32 — 60 lb.
15. 5.32 — vasectomised.
26. 5.32 — 67 lb.
22. 7.32 — 65 lb.
24. 8.32 — 72 lb.
29.12.32 — 107 lb.

TABLE 19.—Sheep 33589.

		14th March, 1932.	18th March, 1932.	23rd March, 1932.	31st March, 1932.	14th April, 1932.	18th April, 1932.	21st April, 1932.	25th April, 1932.	27th April, 1932.	3rd June, 1932.	11th July, 1932.	13th July, 1932.	29th Sept., 1932.
Haemoglobin, gm. per 100 c.c		13·31	14·28	15·71	16·54	13·68	13·31	13·87	16·29	13·87	13·12	14·49	14·08	12·13
Sugar, mg. %	L	78·74	70·92	67·11	62·89	58·14	59·52	62·60	54·94	69·93	63·69	53·76	57·80	55·87
(Glucose)	U	70·92	60·24	56·82	50·76	50·30	49·02	62·50	57·47	59·52	52·63	43·67	43·86	51·55
Total-Nitrogen, gm. N %	L	2·81+	2·926	3·066	3·017	2·926	2·828	2·884	3·054	2·723	2·870	2·905	2·842	2·534
Non-Protein Nitrogen, mg. %	L	22·40	30·16	31·58	30·92	27·26	22·72	21·82	26·08	25·00	17·65	17·04	18·63	16·76
	U	13·32	22·98	22·90	18·38	18·75	12·66	15·00	14·28	14·35	10·71	9·43	11·11	10·60
Coagulable Nitrogen gm. %	L	2·792	2·896	3·034	2·986	2·899	2·805	2·862	3·026	2·698	2·852	2·888	2·823	2·517
	U	2·801	2·903	3·043	2·999	2·907	2·815	2·869	3·038	2·709	2·859	2·896	2·831	2·523
Urea mg. N %	N	7·70	11·34	13·29	8·90	6·14	5 27	4·88	5·78	5·66	3·00	1·63	3·16	1·44
mg. U %	U	16·17	23 8	27·9	18·69	12 81	11 0	1 2	12 0	11·8	6·3	1·40	6 6	3·00
mg. N %	L	7·00	12·99	13·81	8·71	6·07	5·45	4·10	5·78	5·35	3·26	t.l.	t.l.	1·44
mg. U %	U	14·7	27·2	29·00	18 27	12·7	11 4	8·61	12·00	11·2	8	t.l.	t.l.	3·00
"Total"-Creatinine mg. N %	L	2·35	1·86	2·14	2·32	2·41	1·97	1·90	1·90	1·75	2·01	2·28	2·75	2·01
mg. TC %		6·36	5 02	5·76	6·26	6 54	5·32	5·14	5·14	4·70	5 40	6·16	7·44	5·40
...-Creatinine mg. N %	L	1·45	1·49	1 40	—	2·05	1·22	1·22	1·56	1·34	1·75	2·01	2·16	1·60
mg. TC %		3·92	4 0	3 78	—	5 54	3 26	3 26	4 22	3 60	4 70	5 40	5·84	4 32
Uric Acid mg. N %	L	·20	·17	·24	·24	·31	·21	·20	·27	·33	·25	·21	·25	·25
mg. UA %		·60	·50	·73	·71	·93	·62	·59	·81	1·0	·74	·62	·76	·74
mg. N %	U	—	—	·12	·11	·13	·11	—	·12	·19	·14	·07	·10	·09
mg. UA %		—	—	·36	·34	·38	·32	—	·36	·57	41	·22	·30	·28
Amino Acid, mg. N %	L	7 00	7·37	7·78	9·21	6 36	8·24	8·24	7 78	7 78	4·67	5 96	5 51	6·09
	U	4 67	5·18	—	3·89	4·67	5·83	5·38	5·15	5·60	2·72	4·02	4 52	4·49
Rest Nitrogen, mg. N %	L	5 15	9·32	8 13	10 25	11·54	7·03	6·60	10·35	9·48	7·72	6·96	6·96	3·06
	U	0·20	3 32	7 57+	5 67+	5 33	0·05	4·30	1·67	1 87	2·84	2·43	4 33	—

* Includes Amino Acid—N.   † Includes "Total"—Creatinine—N.

History: Born at Onderstepoort 8.2.32, of Ewe 15398 (vide Group A).

Weight: 29.4.32 — 48 lb
15.5.32 — 47 lb.
26.5.32 — 55 lb.
22.7.32 — 57 lb.
24.8.32 —

Excluded from experiment, October 1932.

TABLE 20.—Sheep 33597.

Date		14th March, 1932.	16th March, 1932.	23rd March, 1932.	31st March, 1932.	14th April, 1932.	16th April, 1932.	21st April, 1932.	25th April, 1932.	28th April, 1932.	27th May, 1932.	3rd June, 1932.	11th July, 1932.	13th July, 1932.	29th Sept., 1932.	21st Dec., 1932.	25th Jan., 1933.	30th Jan., 1933.
Haemoglobin gm., per 100 c.c.		12·59	—	14·28	13·31	14·95	12·77	12·77	12·28	10·87	14·28	12·28	11·14	12·42	13·50	13·31	13·87	13·12
Sugar, mg. %	L	74·07	79·36	72·46	67·57	60·24	59·58	—	62·89	66·67	50·50	53·19	49·95	53·48	45·66	45·45	52·63	51·02
(Glucose).	r	63·69	—	66·23	60·61	48·08	54·64	—	60·60	58·82	39·68	45·05	44·25	45·05	40·82	40·82	45·05	40·49
Total-N., gm. N %		2·660	—	2·730	2·709	2·940	2·842	2·730	2·625	2·450	2·905	2·632	2·436	2·478	2·597	2·632	2·751	2·506
Non-Protein Nitrogen, mg. %	L	23·54	25·56	27·40	31·42	27·90	24·10	24·80	24·80	25·64	18·29	23·90	15·00	17·65	17·97	32·42	24·00	30·00
	r	14·16	—	21·43	23·42	20·70	14·63	14·02	15·00	15·15	10·23	13·04	9·31	10·53	9·86	24·58	15·79	23·56
Coagulable Nitrogen, gm. N %	L	2·636	—	2·703	2·678	2·912	2·818	2·705	2·601	2·424	2·887	2·608	2·421	2·460	2·576	2·600	2·727	2·576
	r	2·646	—	2·709	2·686	2·919	2·827	2·716	2·610	2·435	2·895	2·619	2·427	2·467	2·587	2·607	2·735	2·582
Urea — mg. N %	L	9·00	9·31	9·75	8·43	6·78	4·49	5·45	8·47	5·97	2·42	5·25	2·42	2·08	1·86	15·00	6·63	10·44
mg. U %	L	18·9	19·5	20·4	17·70	14·20	9·4	11·4	17·70	12·5	5·04	11·0	5·04	4·26	3·90	31·50	13·86	31·00
mg. N %	r	10·70	—	9·20	9·05	6·78	5·17	4·38	7·16	5·85	2·45	5·66	t.l.	t.l.	1·50	15·74	6·90	9·81
mg. U %	r	22·5	—	19·3	18·9	14·2	10·9	9·2	15·1	12·2	5·04	11·8	t.l.	t.l.	3·15	32·97	14·49	20·58
"Total",—Creatinine — mg. N %	L	2·10	1·78	1·97	2·41	2·39	2·41	1·67	1·36	1·67	2·04	2·16	2·85	2·04	2·11	2·23	2·23	2·29
mg. TC %	L	5·68	4·80	5·42	6·54	6·46	6·54	4·30	4·22	4·50	5·50	5·84	7·72	5·50	5·68	6·00	6·00	6·16
mg. N %	r	1·38	—	1·40	1·78	1·90	1·67	1·67	1·36	1·38	1·60	1·67	2·35	2·01	1·49	2·10	1·89	2·01
mg. TC %	r	3·72	—	3·78	4·80	5·14	4·50	4·30	4·22	3·72	4·32	4·50	6·36	5·40	4·00	5·68	5·14	5·40
mg. N %	L	·27	—	·24	·24	·29	—	—	·21	·26	·22	·21	·23	·23	·09	·43	·34	·29
mg. UA %	L	·80	—	·71	·71	·86	—	·15	·62	·78	·65	·64	·70	·70	·27	1·30	1·03	·86
Uric Acid — mg. N %	r	—	—	·14	·13	·13	—	—	·11	·12	·10	·08	·11	·09	·08	·14	·24	·12
mg. UA %	r	—	—	·41	·38	·38	—	·44	·33	·36	·31	·23	·32	·28	·24	·42	·72	·37
Amino Acid, mg. N %	L	6·86	7·37	8·24	9·33	8·97	8·75	7·0	7·78	7·78	6·36	6·67	5·74	5·64	5·93	7·07	7·00	6·87
	r	4·52	—	—	6·73	5·60	6·09	4·67	5·0	5·71	5·04	4·52	3·33	4·38	4·21	5·26	5·22	5·38
Rest Nitrogen, mg. N %	L	5·31	7·10*	7·20	11·01	9·47	8·45*	10·53	6·78	9·96	7·25	9·61	3·76	7·71	7·80	6·69	7·80	10·11
	r	−2·44	—	10·69*	5·73	6·29	1·70*	3·63	1·37	2·09	1·04	1·11	3·32	4·05	2·58	−0·27	1·54	6·24

*Includes Uric Acid—N. †Includes Amino Acid—N.

History: Born at Onderstepoort, 17.2.32, of Ewe 24158 (vide Group A).

Weight

29. 4.32	— 39 lb.
26. 5.32	— 42 lb.
22. 7.32	— 47 lb.
24. 8.32	— 46 lb.
29.12.32	— 63 lb.

GRAPH II.

Percentage Curves. Nitrogen Partition of Non-Protein Nitrogen.

Group B.
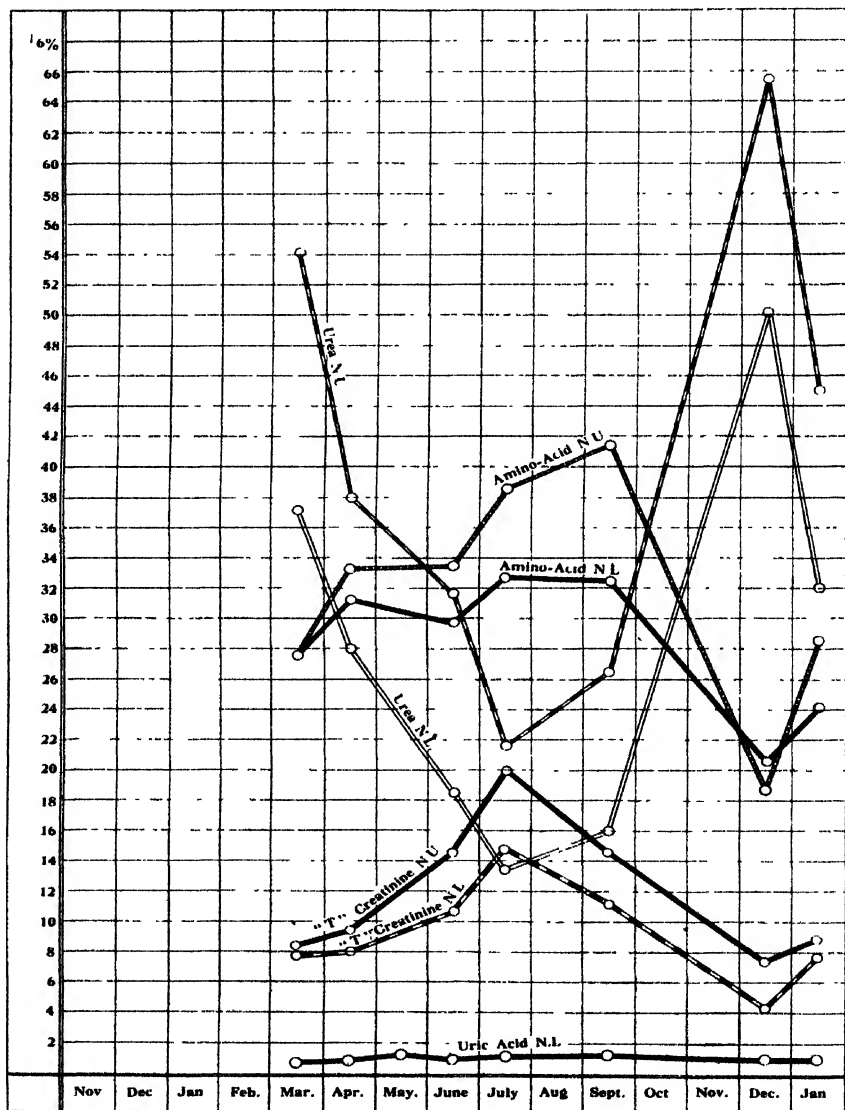
Group B (lambs.)

TABLE 21.—*Haemoglobin (Hb.) gm. per 100 c.c.*

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	15·36	13·30	- -	13·22	13·77	--	11·96	—	—	14·95	14·57
33589....	14·96	14·20	—	13·12	14·28	- -	12·13	—	—	—	—
33597....	13·39	12·73	14·28	12 28	11·78	—	13·50	-	—	13·31	13·49
Average	14·68	13·41	—	12·96	13·28	—	12·53	—	—	14·13	14·03

No conclusion can be drawn from the Hb. figures as regards variation. Attention is, however, drawn to the fact that the figures are higher during March, when the animals were still younger. It should be noted that the Hb. figures were usually found abnormally high or low at the beginning of the experiment.

The Hb. varies from 10·87 to 16·54 gm. % with an average of all the figures of 13·7.

The following table indicates the distribution:—

gm. per 100 c.c.	Occurrence.
Below 11	1
11–12	3
12–13	9
13–14	16
14–15	10
15–16	3
16–17	4

34 % of the determinations lie between 13 and 14 gm. % and 76 % between 12 and 15.

TABLE 22.—*Sugar mg. % ("Laked")*.

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208... .	51 3	53·0	—	61 3	48·2	—	59 2	—	—	49·7	45·6
33589 ...	69·9	63·0	—	63·7	55·8	- -	55·7	—	—	—	—
33597 ...	73·4	59·2	50 5	53 2	51·7	-	45 7	—	—	45·4	51·8
Average	64 8	57·9	- -	58·7	51·5	--	51 6	—	—	47·6	48·7

TABLE 23.—*Sugar mg. % ("Unlaked")*.

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	46·9	51·1	—	56·7	43·9	—	51·0	—	—	45·7	39·6
33589....	59·7	55·8	—	52·6	43·8	—	51·5	—	—	—	—
33597....	63·5	55·5	39 6	45 1	44·6	—	40·8	—	—	40·8	42·8
Average	56·1	54·2	—	52·8	44·1	—	47·8	—	—	43·2	41·2

" Laked " Filtrates.

Minimum-maximum variation, 42·7–79·4 mg. %.
Average, 57·6 mg. %.

" Unlaked " Filtrates.

Minimum-maximum variation, 35·8–70·9 mg. %.
Average, 50·8 mg. %.
Average difference, 6·8 mg. %.

The following table indicates the distribution:—

" Laked " Filtrates.		" Unlaked " Filtrates.	
mg. %.	Occurrence.	mg. %.	Occurrence.
40–45	2	35–40	3
45–50	9	40–45	9
50–55	13	45–50	11
55–60	7	50–55	8
60–65	7	55–60	7
65–70	5	60–65	6
70–75	3	65 and over	2
75–80	2		

24·5 % of the determinations of the " laked " lie between 50 and 55 mg. % and 55 % between 50 and 65, while 24 % of the determinations of the " unlaked " filtrates lie between 45 and 50 and 61 between 40 and 55 mg. %.

Comparison.

The differences of the " laked " and " unlaked " figures vary from 3·3 to 20·6 % with an average of 11·8.

From March to July the blood sugar level fell heavily but was still much higher in comparison with that of Group A (see Graph III).

The tendency of the sugar level to rise towards winter with this given ration, triumphed even over this observed tendency to decrease with age to reach the normal figure of adult sheep. This is even more evident if the average of the different lambs on Tables 22 and 23 are compared. S. 33208 was 10 weeks old at the beginning of the analyses and throughout the blood sugar level increased towards June.

S. 33587 was 5 weeks old and the level remains constant from April to June, from where it decreases again.

S. 33597 was 4 weeks old and the tendency to decrease triumphed wholely over the seasonal inclination towards a rise.

The change in the ration in December, 1932, did not cause a great variation (" Laked " December, 1932, 47·6, January, 1933, 48·7).

The percentage differences in March and June are about equal (13 and 10, see Graph III and c.f. Tables 22 and 23).

Total Nitrogen.

Minimum-maximum variation, 2·4–3·1 gm. N %.

Average, 2·8 gm. N %.

The total nitrogen remains fairly constant. C.f. the tables for individual differences (Tables 18-20).

S. 33597 is exceptionally low (2·66, 2·73, 2·70, 2·94, etc.). S. 33208 being higher (3·066, 3·024, 3·038, etc.).

TABLE 24.—*Non-Protein Nitrogen mg. %. " Laked ".*

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	23·6	20·3	—	15 3	15·5	—	18·1	—	—	32·6	26·1
33289....	28 8	24 6	—	17 6	17 8	—	16·8	--	—	—	—
33297....	27·0	25·5	18 3	23·9	16·3	—	18·0	--	—	32·4	27·0
Average	26 5	23·5	—	18 1	16·5	—	17·6	—	—	32·5	26·5

TABLE 25.—*Non-protein Nitrogen mg. % (" Unlaked ").*

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208. ...	15 8	15·3	—	10·6	10 6	--	11·4	—	—	25·9	18·0
33289. ..	19 4	15·0	—	10 7	10·3	- -	10 7	—	—	—	—
33297 ...	19 7	15·4	10 2	13 0	9·9	—	9 9	—	—	24·6	19·7
Average	18·1	15 4	—	11 2	10 3	–	10 6	—	—	25·2	18·8

" Laked " Filtrates.

Minimum-maximum range, 14·8–32 4 mg. N %.

Average, 23·0 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum range, 9·3–25· mg. N %.

Average, 15·0 mg. N %.

Average difference, 8·0 mg. N %.

The following table indicates the distribution :—

" Laked " Filtrates.

mg. %.	Occurrence.
Below 15	1
15–20	16
20–25	13
25–30	9
30–35	7

" Unlaked " Filtrates.

mg. %.	Occurrence.
Below 10	4
10–15	23
15–20	9
20–25	9
Above 25	1

" Laked " Filtrates.

35 % of the determinations lie between 15 and 25 mg. N %.

83 % of the determinations lie between 15 and 30 mg. N %.

" Unlaked " Filtrates.

50 % of the determinations lie between 10 and 15 mg. N %.

70 % of the determinations lie between 10 and 20 mg. N %.

Comparison.

The difference between the " laked " and " unlaked " figures varies from 23·2 to 45 % with an average of 35 %, with an average difference of 8·0 in mg. N % (23–15).

From Graph IV it is evident that the percentage differences between the " laked " and "unlaked " figures increase towards the winter since the curves run about parallel (decreasing) and consequently the differences in mg. N % remain about the same.

Note the individual differences of the three lambs, S. 33208 being the lowest (23·6, 20·3, 15·5, etc.), and S. 33297 the highest (27·0, 25·5, 18·3, 23·9) (*vide* Tables 24 and 25).

TABLE 26.—*Urea Nitrogen mg. % (" Laked ").*

Sheep No	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	10·2	5·9	—	2 6	2·1	—	3·8	—	—	17·7	8·5
33589...	10·3	5 6	· ·	3·0	2·4	· ·	—	—	—	· —	—
33597....	9·1	6 2	2·4	5·2	2 2	—	1 9	—	—	15 0	8·0
Average	9·8	5·9	–	3·3	2·2	—	2 8	—	—	16·3	8·5

The " laked " urea nitrogen varies for an undeterminable small quantity (below 1·5 mg. N %) to 17·69 mg. N % with an average of 6·6 mg. N %.

The following table indicates the distribution :--

mg. N %.	Occurrence.
Below 1·5 ... ... ,..	1
1–2	2
2–2	2
3–4	5
4–5	3
5–6	7
6–7	4
Above 7	18

The urea nitrogen decreased gradually from the beginning of the analyses towards winter. In December (1932) the maximum level was reached, but after the withdrawal of the green fodder from the ration it decreased again immediately.

In the case of the " unlaked " the same applies here as in Group A and by comparison of the " laked " and " unlaked " figures on Tables 18-20 it is seen by what quantities the figures differ. The " unlaked " are usually the lowest. In 5 instances the urea nitrogen was too low to be determined (less than 1·5 mg. % N).

The maximum figure obtained for the " unlaked " filtrates is 18·05 mg. N %.

Comparison.

The urea nitrogen curve (see Table 26 and Graph IV) runs approximately parallel with the two non-protein nitrogen curves (*vide* Tables 24 and 25) of Group B. Since the urea nitrogen and non-protein nitrogen curves run parallel, although of course the urea nitrogen is obviously on a much lower level, it is evident that the urea nitrogen percentage of the N.P.N. (both "laked" and "unlaked") fall heavily, during the period of March to July (see Graph II and Table 26). It again increases to December, 1932, and decreases towards January, 1933. On Graph I two urea nitrogen percentage curves are given (see explanation at the end of Urea N under small heading "Comparison" in Group A).

The "unlaked" urea N percentage curves run much higher than the "laked" because the respective N.P.N. is much less.

The individual differences are not very striking.

TABLE 27.— " *Total* " *Creatinine Nitrogen mg. %* " *Laked* ".

Sheep No.	March	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	1 92	1 70	---	1·84	2·37	—	1 82	—	—	1·90	1·85
33589....	2·17	1·98		2·01	2·51	· ·	2·01	—	--		2·26
33597...	2·06	1 94	2 04	2·16	2 44	---	2·11	—	---	2·23	2·26
Average	2 05	1 88	---	1 96	2 44	—	1·98	—	—	2·06	2·05

TABLE 28.—" *Total* " *Creatinine Nitrogen mg. %* " *Unlaked* ".

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208 .	1·49	1 48	–	1 52	1 90	—	1 45	--	—	1·60	1·41
33589 . .	1·45	1 45	--	1·75	2·08	—	1 60	-·	—	—	—
33597	1 52	1 52	1 60	1 67	2 18	-·	1 49	· ·	—	2·10	1·95
Average	1·48	1 48	---	1·62	2 06	—	1·55	--·	—	1·85	1·6ʰ

" *Laked* " *Filtrates.*

Minimum-maximum variation, 1·56–2·85 mg. N %.
Average, 2·04 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, 1·22–2·35 mg. N %.
Average, 1·59 mg. N %.
Average difference, ·55 mg. N % (laked to unlaked.)

Comparison.

The difference between "laked" and "unlaked" "total" creatinine nitrogen lie between 0 and 40 % (see Table 18, 23.3.32. L 1·56 U. 1·67) and the average difference is 23%.

The curves in mg. N % are not given on Graph IV because they would coincide closely with the respective curves of Group A and have been omitted for the sake of clearness.

The differences in per cent. decrease towards winter (27–17 %).

On Graph II the percentage curves rise considerably, contrary to the urea N percentage curves, of which the " laked " is lower in July than the " T ".C.N. (13·6 %). The " laked " " T ".C.N. curve rises from 8 to 15 %, and the " unlaked " from 8 to 20. That the percentage amounts do not increase equally is clear as the curves approach each other (Graph IV) and the non-protein nitrogens run approximately parallel.

Note that the individual differences are in all cases relatively small (On Table 28, S. 33208, 1·92, 1·70, 1·84, etc., S. 33589, 2·17, 1·98, 2·01, etc.).

TABLE 29.—*Uric Acid Nitrogen mg.* % " *Laked* ".

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208 ...	·16	·15	—	·15	·14	—	·18	- -	- -	·23	·23
33589 ...	·21	·26	—	·25	·23	—	·25	--	--	—	—
33597....	25	·23	·22	·21	·23	—	·27	—	-	43	·32
Average	·20	·22	—	·19	·20	—	23	—	—	·33	·27

" *Laked Filtrates.*

Minimum-maximum variation, less than ·10–·43 mg. N %.
Average, ·20 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, less than ·10–·24 mg. N %.
As in Group A no Table of the " unlaked " is given.

Comparison.

In respect of the changeability of the proportions of the " laked " to the " unlaked " figures the same applies here as in Group A, namely, that not only are the figures very changeable, but so is also the relation of the figures of the two filtrates (e.g. Table 18, 14.3.32. L ·23, U. t.l., and 25.4.32, L ·13, U ·13 etc.).

No curves are drawn of the uric acid nitrogen on Graph II and IV because the " laked " one would practically coincide with the given uric acid curve of Group A, and the " unlaked " one has been omitted owing to the incomplete range of figures available, as this constituent in " unlaked " filtrate was frequently below the range of accurate determination. The " laked " curve (in mg. N %) would run about in a straight line on ·2 mg. N % level (Table 29) and the percentage (uric acid nitrogen) of the respective N.P.N. rises from ·8 to 1·2 % from March to July. It is of interest to note the individual low figures encountered, e.g. on Tables 29, S. 33208 never rises above ·23 while the lowest of S. 33597 is ·21 with a maximum of ·43 mg. N %.

TABLE 30.—*Amino-Acid Nitrogen mg. %, " Laked ".*

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	6·21	6·16	—	5·10	4 74	—	5·15	--	—	6·36	5·93
33589....	7·84	7·78	—	4·67	5 73	—	6·09	—	—	—	—
33597....	7 95	8 06	6·36	6·67	5·69	—	5·93	—	...	7·07	6·93
Average	7·33	7 55	—	5·38	5·39	-	5 72	—	—	6·71	6 43

TABLE 31.—*Amino-Acid Nitrogen mg. %, " Unlaked ".*

Sheep No.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
33208....	5 07	5·05	-	3 92	3 76	—	4 52	—	—	5 26	5 49
33589....	4 58	5 32	—	2 72	4 26	—	4 49	—	...	—	- -
33597 ...	5 62	5·41	5·04	4 52	3 85	-	4 21	--	—	5 26	5 30
Average	5 03	5 26	· ·	3 77	3 96	—	4 40	—	—	5 26	5·39

" Laked " Filtrates.

Minimum-maximum variation, 4 67–9·33 mg. N %.
Average, 6 67 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, 2·72–6·73 mg. N %.
4·60 mg. N %.
Average difference, 2·07 mg. N % (laked to unlaked.)

Comparison.

The difference of the " laked " and " unlaked " figures varies from 7·7 % to 58 % with an average of 31 % (2·07 mg.).

The curves in mg. N % have the inclination to approach each other towards winter (c.f. Graph IV and Tables 30 and 31). In March the difference was 2·3 mg. N % and in July only 1·43 at the expense of the " laked " figure. Both curves fall to a minimum in June and rise again after that period, the " laked " figures not approaching, however, the same level in December (1932) as in March, contrary to the rise of the " unlaked " figures (L. 7·3, 7·3, 5·4, 5·4, 6·7, 6·4 and U 5·0, 5·3, 3·8, 4·0, 5·3, 5·4). Note the fall of the percentage curve in December (1932) and the rise in January (1933) which may possibly, if not wholly, be due to the change of rations introduced during this period (Graph II). The difference between the " laked " and " unlaked " figures in March is 31 % and 27 % in July.

On Graph II the percentage curves rise considerably and are fairly parallel, because though the averages fall considerably from March to June (Tables 30 and 31 L. 7·3, 7·3, 5·4 and U. 5·0, 5·3, 3·8 respectively, they do not do so proportionally to the respective non-protein nitrogens.

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(c) Explanation of Graphs III and IV.

In order to compare the changes of the blood sugar content (both " laked " and " unlaked ") in blood of the two Groups A and B, curves have been plotted on the same paper, with the average of all the respective figures of a group per month being recorded as points in mg. glucose per cent. (Graph III). Such curves of all the other constituents (except T.N. and Hb.) of Groups A and B in mg. N % have been recorded on the same graph (Graph IV).

The tables from which these graphs (III and IV) have been plotted together with the same tables of Groups C, D and E and also of the Hb. are all stated in the discussion associated with each separate constituent (Tables 7-17, 21-31, 35-45, 52-62, 68-78).

The averages for each sheep per month are also given in these Tables in order to be able also to note any individual differences between the sheep in a group.
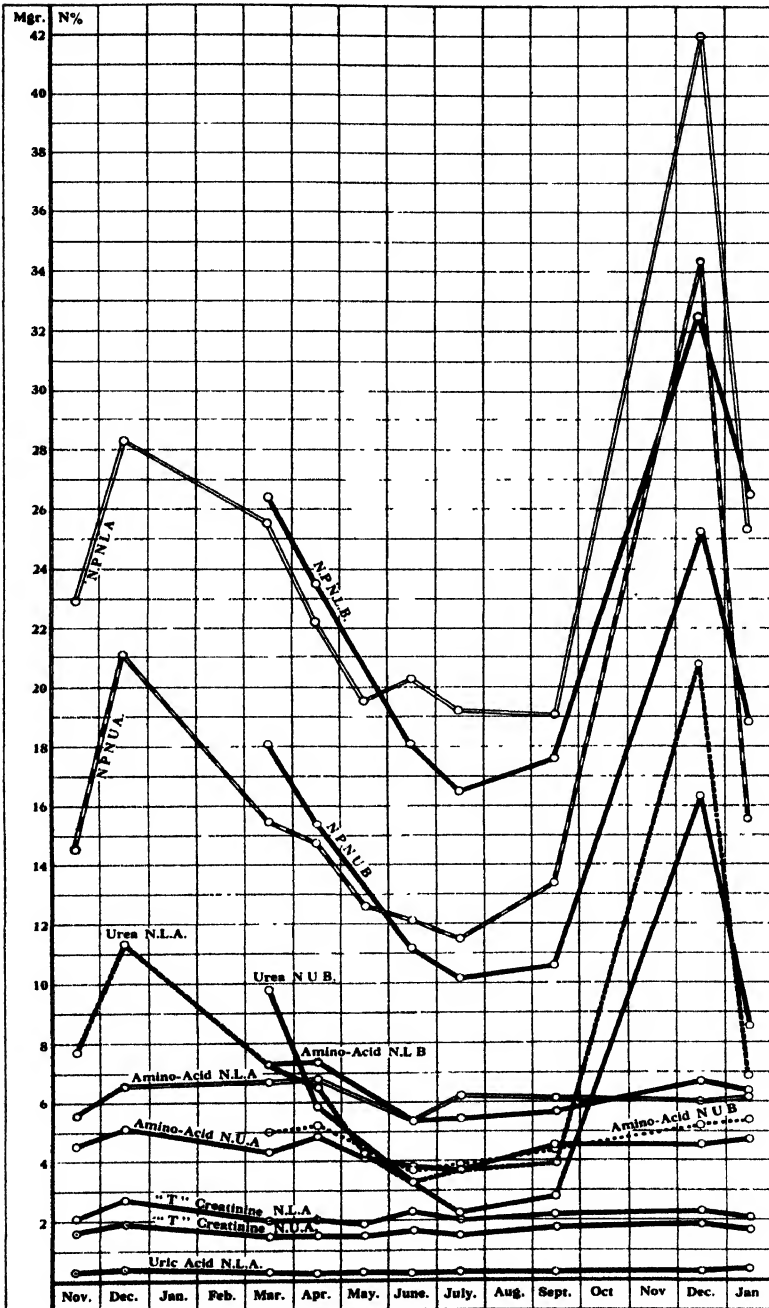
Graph III.

Blood Sugar Curves in mg. %.

Groups A and B.
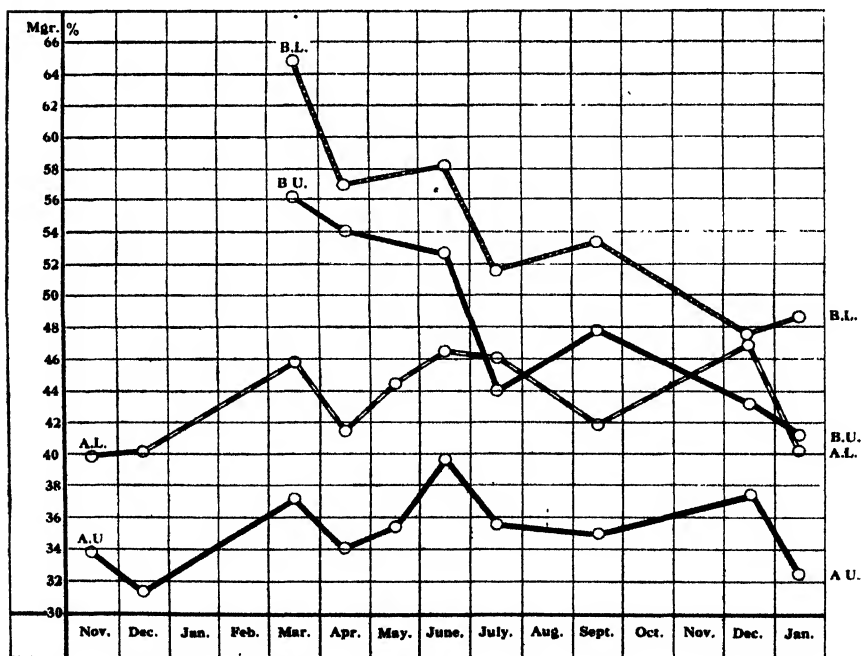
GRAPH IV.
Nitrogen Partition in mg. N %.
Groups A and B.

(d) GROUPS C, D AND E.

As the curves of Groups C, D and E show the same general tendencies as Groups A and B they are not given here. I have, however, for the sake of completeness recorded here the full analytical data gathered. These have been more briefly summarized in order to avoid needless repetition. All the other data, e.g. ranges between which the constituents vary and averages, etc., are also included.

GROUP C.

Sheep No. 22204, Table 32.
Sheep No. 25140, Table 33.
Sheep No. 25142, Table 34.

TABLE 32.—Sheep 22204.

Date.		11th Nov., 1931.	13th Nov., 1931.	2nd Feb., 1932.	4th Feb., 1932.	22nd Feb., 1932.	26th Feb., 1932.	3rd March, 1932.
Haemoglobin gm. per 100 c.c		12·42	11·96	15·19	17·18	14·49	—	13·87
Sugar (Glucose) mg. %..	L	34·84	43·67	56·18	38·46	48·78	37·04	43·10
	U	31·45	39·22	46·73	23·25	43·57	32·79	39 68
Total N, gm. N %......		2·730	2·660	2·954	2·996	3·080	3·075	3·010
Non-protein Nitrogen mg. %	L	21·27	32·08	30·00	35·30	22·22	33·32	25·64
	U	15·15	26·42	24·00	30·92	15·79	27·36	17·65
Coaguable Nitrogen gm.N.%	L	2·709	2·628	2·924	2·961	3·058	3·042	2·984
	U	2·715	2·634	2·930	2·965	3·064	3·048	2·992
mg. N %.............	L	5·24	5·25	6·72	20·78	7·73	18·42	7·33
mg. U %.............	L	11·0	11·0	14·07	43·60	16·20	38·64	15·40
Urea								
mg. N %.............	U	6·79	14·43	16·40	—	7·62	17·27	7·33
mg. U %.............	U	14·20	30·30	34·49	—	15·96	36·20	15·40
mg. N %.............	L	2·48	2·55	1·97	2·33	2·25	2·23	1·89
mg. TC %.............	L	6·7	6·86	5·32	6·26	6·12	6·00	5·14
" Total " Creatinine								
mg. N %.............	U	1·78	2·19	1·82	2·11	1·75	1·89	1·41
mg. TC %.............	U	4·80	5·86	4·90	5·68	4·70	5·14	3·80
mg. N %.............	L	·52	·33	·25	·18	·26	·23	·19
mg. UA %.............	L	1·55	1·0	·74	·53	·78	·68	·57
Uric Acid								
mg. N %.............	U	—	·19	·15	—	·17	·15	·10
mg. UA %.............	U	—	·57	·44	—	·52	·45	·29
Amino-Acid mg. N %..	L	5·00	5·18	6·67	5·38	5·98	6·10	6·36
	U	4·00	4·24	4·35	—	4·38	4·83	5·00
Rest Nitrogen mg. N %..	L	8·03	8·77	4·39	6·63	6·00	6·34	9·87
	U	2·58	5·37	1·28	—	1·87	3·22	3·81

History : 24.2.30 Helminthiasis.
18.5.32 Bluetongue.
Weight : 18.12.31—67 ℔.
22.1.32—80¼ ℔.
23.2.32—76 ℔.
29.4.32—85 ℔.
Discharged : 29.4.32.

Date.		12th Nov., 1931	18th Nov., 1931.	20th Nov., 1931.	5th Feb., 1932.	10th Feb., 1932.	16th Feb., 1932.	19th Feb., 1932.	22nd Feb., 1932.	26th May, 1932.	1st June, 1932.	9th June, 1932.	28th June, 1932.	29th June, 1932.	28th Sept., 1932.	15th Dec., 1932.	24th Jan., 1933.	30th Jan., 1933.
Haemoglobin, gm. per 100 c.c.		11·96	14·28	12·42	17·18	15·42	15·42	14·72	15·42	12·94	16·87	17·18	18·20	14·08	15·98	23·18	21·06	18·57
Sugar, mg.%		37·17	56·82	30·50	46·87	45·45	43·48	42·19	35·91	73·56	50·30	42·19	40·49	47·17	43·48	47·37	39·06	38·46
(Glucose)		34·48	39·68	44·05	29·41	29·15	33·67	26·25	28·01	60·98	41·32	30·49	32·26	45·05	35·71	33·00	29·24	25·00
Total N, gm. N%		2·688	2·800	2·492	3·168	3·183	3·024	3·094	3·122	2·884	3·136	3·255	3·311	2·884	2·982	3·274	3·304	3·157
Non-Protein Nitrogen, mg.%	L	31·90	26·20	23·06	25·96	25·20	31·90	—	25·98	20·98	24·00	—	—	20·98	19·86	39·46	25·98	26·08
	U	23·06	20·56	16·48	20·98	17·14	21·58	—	15·31	11·32	13·00	—	10·71	12·14	12·93	27·36	14·76	15·79
Coaguable Nitrogen, gm. N%	L	2·656	2·774	2·469	2·139	3·158	2·992	—	3·096	2·861	3·112	—	—	2·863	2·962	3·235	3·278	3·131
	U	2·665	2·779	2·476	2·147	3·166	3·002	—	3·107	2·873	3·123	—	3·300	2·872	2·969	3·247	3·289	3·141
Urea. mg. N%	L	7·12	13·19	11·30	9·93	8·17	15·25	—	7·70	4·33	5·55	—	2·64	1·0	6·69	15·25	4·71	6·15
mg. U%	L	14·98	27·60	24·15	20·80	17·15	32·00	—	16·17	9·10	11·60	—	5·30	2·10	14·07	31·92	9·47	12·81
mg. N%	U	7·12	13·14	10·77	10·24	8·17	14·88	—	7·47	4·13	4·79	—	2·31	1·13	6·50	13·81	·15	6·02
mg. U%	U	14·95	27·60	22·60	21·50	17·15	31·20	—	15·65	8·70	9·80	—	5·25	2·16	13·65	28·98	·44	12·60
"Total" Creatinine. mg. N%	L	—	2·48	2·11	2·23	2·04	2·15	1·93	2·04	1·82	2·15	1·86	2·59	1·82	2·35	2·48	2·11	2·11
TC%	L	—	6·74	5·00	6·00	5·54	5·76	5·18	5·34	4·90	5·84	5·02	6·96	4·90	6·36	6·74	5·68	5·68
mg. N%	U	—	1·60	1·45	1·73	1·56	1·82	1·75	1·38	1·41	1·67	1·67	2·23	1·86	1·82	2·01	1·56	1·75
TC%	U	—	4·36	3·86	4·70	4·16	4·84	4·70	3·78	3·86	4·70	4·50	6·00	5·02	4·80	5·40	4·16	4·70
Uric Acid. mg. N%	L	·31	·37	·25	·27	·24	·32	·32	·25	·25	·28	·29	·33	—	·27	·20	·46	·88
mg. UA%	L	·93	1·12	·76	·82	·73	·97	·93	·75	·75	·85	·87	1·00	—	·80	·59	1·39	1·14
mg. N%	U	—	—	·12	·14	·12	·17	·17	·09	·12	·13	·17	·16	—	·09	·10	·15	·11
mg. UA%	U	—	—	·35	·41	·35	·52	·52	·26	·37	·40	·50	·49	—	·28	·30	·44	·34
Amino-acid mg. N%	L	7·78	5·71	5·96	5·60	6·67	7·78	7·14	7·00	6·06	5·76	6·06	5·76	5·60	6·63	5·98	7·87	7·00
	U	7·00	4·67	5·28	4·12	4·24	5·60	5·30	5·18	4·11	2·80	2·91	3·73	4·83	3·68	4·27	6·09	5·47
Rest Nitrogen, mg. N%	L	16·69†	4·45	3·24	11·15	8·08	6·40	—	8·99	11·21	10·29	—	—	12·36*	3·91	15·95	10·83	10·44
	U	8·94†	1·15	-1·14	4·73	3·17	·77	—	1·19	1·55	3·53	—	2·08	4·32*	·84	6·17	—	2·44

† Includes "Total" Creatinine-N.
* Includes Uric Acid-N.

History : 12. 9.29 Helminthiasis.

Weights :
18.12.31 — 87 ℔
22. 1.32 — 97½ ℔
23. 2.32 — 95¾ ℔
29. 4.32 — 107 ℔
26. 5.32 — 106 ℔
22. 7.32 — 110 ℔
24. 8.32 — 116 ℔
29.12.32 — 118 ℔

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TABLE 34.—Sheep 25142.

Date.		13th Nov., 1931.	19th Nov., 1931.	25th Nov., 1931.	2nd Feb., 1932.	4th Feb., 1932.	15th Feb., 1932.	18th Feb., 1932.	15th April, 1932.	19th April, 1932.	17th May, 1932.
Haemoglobin, gm. per 100 c.c.		12·94	—	12·79	17·51	18·20	15·19	13·50	12·59	15·42	14·28
Sugar, mg. %	L	47·39	44·64	43·48	47·17	38·46	65·50	47·17	58·85	—	44·84
(Glucose)	U	43·48	35·99	38·46	34·48	33·90	41·84	40·82	53·19	—	34·48
Total N, gm. N %		2·870	2·968	2·814	3·099	3·052	3·030	3·010	3·010	3·234	3·108
Non-Protein N, mg. %	L	—	—	—	28·70	31 58	25 86	30·30	21·82	28·98	24·40
	U	—	—	—	21·58	23 06	17·65	22·98	15 55	16·94	13·45
Coaguable Nitrogen, gm. N %	L	—	—	—	3 070	3·020	3·004	2 980	2·988	3·205	3·084
	U	—	—	—	3·077	3·029	3 012	2·987	2·994	3·217	3 095
Urea. mg. N %	L	—	—	—	14·50	17·69	8·56	12·36	5·85	9 93	5·55
mg. U %	U	—	—	—	30 45	37 00	17·90	25·90	12·25	20 80	11·60
"Total" Creatinine mg. N %	L	2·89	2·66	2·40	2·36	2 33	2 33	2 04	2·04	1·49	1·67
mg. TC %		7·78	7 20	6 54	6·40	6·00	6·26	5 54	5 54	4·00	4·50
mg. N %	U	2 19	2·23	1·82	1 89	2 01	1 93	1·93	1·65	1·3	1·78
mg. TC %		5 86	6·00	4 90	5·14	5·40	5·24	5·14	4·22	3·40	4·80
Uric Acid mg. N %	L	—	—	—	·25	·30	·42	·34	·28	·24	·29
mg. UA %		—	—	—	·75	·89	1 25	1·02	·84	·72	·86
mg. N %	U	—	—	—	·15	—	—	·17	·15	—	·11
mg. UA %		—	—	—	·44	—	—	·52	·44	—	·33
Amino Acid, mg. N %	L	6 22	—	6·36	6·09	4·67	7·37	6 36	7·45	7·00	6·73
	U	—	—	4·67	3·50	3·89	5·38	4·67	4·67	4·83	4·00
Rest Nitrogen, mg. N %	L	—	—	—	5·50	6 69	7·18	9·20	6·20	10·32	10·16
	U	—	—	—	1·58	0·46	1·44	3·51	3·51	1·08	1·37

History :   24. 2.30 Helminthiasis.
Weight :
18.12.31 — 93 lb.
22. 1.32 — 102¼ lb.
23. 2.32 — 100 lb.
29. 4.32 — 115 lb.
29. 4.32 — discharged

GROUP C. (*Three six-tooth ewes*).
TABLE 35.—*Haemoglobin gm. per 100 c.c.*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	12·2	—	—	15 6	13 9	—	—	—	—	—	—	—	—	—	—
25140	12·9	—	—	15·6	—	—	12 9	16 6	—	—	16 0	—	—	23·2	19·8
25142	12·9	—	—	16 1	—	14 0	14 3	—	—	—	- -	—	—	—	—
Av...	12·7	—	—	17 8	13 9	140	14 0	16 6	---	—	16 0	—	—	23 2	19·8

Minimum-maximum variation in gm. per 100 c.c. 11·96–23·18.
Average, 16·7.

The following table indicates the distribution:—

gm. per 100 c.c.	No. of determinations.
10–11	0
11–12	2
12–13	6
13–14	2
14–15	5
15–16	7
16–17	1
17–18	3
18 and more	5

21 % of the determinations lie between 15 and 16 gm. per 100 c.c. and 50 % between 13 and 15 gm. per 100 c.c.

TABLE 36.—*Sugar mg. % (" Laked ").*

Sheep No.	Nov	Dec	Jan.	Feb	Mar	Apl	May.	June	July	Aug.	Sept	Oct.	Nov.	Dec.	Jan
22204	39 2	—	—	45 1	43 1	- -	—	73 5	45 1	—	- -	—	—	47 4	38·8
25140	48 2	—	—	13 4	—	—	—	73 5	45 1	—	43 4	—	—	47 4	38·8
25142	45 2	—	—	49 6	- -	58 8	44 8	—	—	—	—	—	—	—	—
Av...	44 8	- -	—	45 8	43 1	58 8	60 7	45 1	—	—	43·4	—	—	47·4	38·8

TABLE 37.—*Sugar mg. % (" Unlaked ").*

Sheep No.	Nov	Dec.	Jan.	Feb.	Mar.	Apl.	May	June	July.	Aug.	Sept	Oct	Nov.	Dec.	Jan.
22204	35·3	—	—	36·6	39 7	—	—	—	—	—	—	—	—	—	27·1
25140	39 4	—	—	27·7	—	—	61 0	37·3	—	- -	35 7	-	—	33 0	27·1
25142	39.3	—	—	37·8	—	53 2	34 5	—	—	—	—	- -	—	—	—
Av...	38 3	—	—	34 3	39 7	53 2	47 7	37·3	—	—	35 7	---	—	33 0	27·1

" *Laked* " *Filtrates.*
Minimum-maximum variation, 34–74 mg. %.
Average, 46·2 mg. %.

" *Unlaked* " *Filtrates.*
Minimum-maximum variation, 23–61 mg. %.
Average, 36·8 mg. %.
Average difference, 9·4 mg. % (laked to unlaked.)

The following Table indicates the distribution : —

"*Laked*" Filtrates.		"*Unlaked*" Filtrates.	
mg. %.	*Occurrence.*	*mg. %.*	*Occurrence.*
25–30	0	20–25	1
30–35	1	25–30	6
35–40	7	30–35	10
40–45	10	35–40	6
45–50	8	40–45	6
50–55	2	45–50	2
55–60	3	50–55	1
60–65	0	55–60	0
65–70	1	60–65	1
70–75	1		

" *Laked* " *Filtrates.*

30 % of the determinations lie between 40 and 45 mg. %.
76 % of the determinations lie between 35 and 50 mg. %.

" *Unlaked* " *Filtrates.*

30 % of the determinations lie between 30 and 35 mg. %.
85 % of the determinations lie between 25 and 45 mg. %.

The differences between " laked " and " unlaked " vary between 40 % and 9%.

The average difference is 20 %.

Total Nitrogen.

Minimum-maximum variation, gm. N % 2·4–3·3.
Average, 2·9 gm. N %.

TABLE 38.—*Non-Protein Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June	July.	Aug	Sept.	Oct.	Nov.	Dec.	Jan.
22204	22·2	—	—	30 6	25 6	—	—	—	—	—	—	—	—	—	—
25140	27·1	—	—	28·0	—	—	21 0	22 5	—	—	19 9	—	—	39 5	26·0
25142	—	—	—	29·1	—	25·4	24·4	—	—	—	—	—	—	—	—
Av...	26·9	—	—	29 9	25·6	25 4	22·7	22·5	—	—	19·9	—	—	39·5	26·0

TABLE 39.—*Non-Protein Nitrogen mg. % (" Unlaked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug	Sept.	Oct.	Nov.	Dec.	Jan.
22204	20·8	—	—	24·5	17·8	—	—	—	—	—	—	—	—	—	—
25140	20·0	—	—	18·7	—	—	11·3	11·9	—	—	12·9	—	—	27·4	15 3
25142	—	—	—	21 3	—	16 2	13 4	—	—	—	—	—	—	—	—
Av...	20·3	—	—	21·5	17 6	16·2	12·9	11·9	—	—	12·9	—	—	27·4	15·3

" Laked ".
Minimum-maximum variation, 19·86–39·06 mg. N %.
Average, 26 mg N %.

" Unlaked ".
Minimum-maximum variation 10·7–27·36 mg. N %.
Average, 19 mg. N %.
Average difference, 7·0 mg. N % (laked to unlaked.)
The following Table indicates the distribution :—

" Laked " Filtrates.		" Unlaked " Filtrates.	
mg. N %.	Occurrence.	mg. N %	Occurrence.
Below 20	1	10–14	7
20–25	8	15–20	10
25–30	10	20–25	11
30–35	8	more than 25 ...	1
more than 35 ...	1		

" Laked " Filtrates.
36 % of the determinations lie between 25 and 30 mg. N %.
64 % of the determinations lie between 25 and 35 mg. N %.

" Unlaked " Filtrates.
34 % of the determinations lie between 15 and 20 mg. N %.
73 % of the determinations lie between 10 and 25 mg. N %.

The percentage differences of the " laked " and " unlaked " figures vary from 12 to 48 with an average of 27%.

TABLE 40.—Urea Nitrogen mg. % (" Laked ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	10·2	—	—	15 9	7 3	—	—	—	—	—	—	—	—	15 3	—
25140	10·6	—	—	10 3	—	—	4 3	3 0	—	—	6·7	—	—	15 3	5·4
25142	—	—	—	13 3	—	7 9	5 5	—	—	—	—	—	—	—	—
Av...	10·4	—	—	13 1	7 3	7·9	4 9	3·0	—	—	6 7	—	—	15 3	5 4

" Laked ".
Minimum-maximum variation, below ·15–20.78 mg. N %.
Average, 9·83 mg. N %.

" Unlaked ".
Minimum-maximum variation, below ·15–17·24 mg. N %.

The following Table indicates the distribution :—

Below 5	3
5–6	4
6–7	2
7–8	4
8–9	2
9–10	2
above 10	11

36 % of the determinations of the " laked " filtrates lie between 5 and 8 mg. N %.

TABLE 41.—" *Total* " *Creatinine Nitrogen mg. %* (" *T* ".*C.N.*) " *Laked* ".

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	2·51	—	—	2·19	1·89	—	—	—	—	—	—	—	—	2·48	—
25140	2·30	—	—	2·04	—	—	1·82	2·10	—	—	2·35	—	—	—	2·11
25142	2·65	—	—	2·24	—	1·76	1·67	—	—	—	—	—	—	—	—
Av...	2·51	—	—	2·19	1·89	1·76	1·74	2·10	—	—	2·35	—	—	2·48	2·11

TABLE 42.—" *Total* " *Creatinine Nitrogen mg. %* (" *T* ".*C.N.*) " *Unlaked* ".

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	1·98	—	—	1·89	1·41	—	—	—	—	—	—	—	—	2 01	—
25140	1·52	—	—	1·65	—	—	1·41	1·88	—	—	1·82	—	—	—	1·66
25142	2 08	—	—	1·94	—	1·45	1·78	—	—	—	—	—	—	—	—
Av...	1·89	—	—	1·79	1·41	1·45	1·59	1·88	—	—	1·82	—	—	2·01	1·66

" Laked " Filtrates.

Minimum-maximum variation, 1·49–2·66 mg. N %.
Average, 2·17 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, 1·34–2·23 mg. N %.
Average, 1·79 mg. N %.
Average difference, 0·38 mg. N %.

The differences between " laked " and " unlaked " " T ".C.N. vary from 7·5 to 35·5 %, with an average of 17·5 %.

TABLE 43.—*Uric Acid Nitrogen mg. %* (" *Laked* ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	·42	—	—	·23	·19	—	—	—	—	—	—	—	—	—	—
25140	31	—	—	·28	—	—	·25	22	—	—	27	—	—	20	·42
25142	—	—	—	·33	—	·26	·29	—	—	—	—	—	—	—	—
Av...	·35	—	—	·28	—	·26	·27	·22	—	—	·27	—	—	·20	·42

" Laked " Filtrates.

Minimum-maximum variation, less than ·10–·52 mg. N %.
Average, 0·29 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, less than ·10–.·19 mg. N %.

TABLE 44.—*Amino-Acid Nitrogen mg. % (" Laked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	5·09	—	—	6·03	6·36	—	—	—	—	—	6 63	—	—	—	—
25140	6·48	—	—	6·84	—	—	6·06	5·79	—	—		—	—	5 98	7·43
25142	6·29	—	—	6 12	—	7·22	6·73	—	—	—	—	—	—	—	—
Av...	6·03	—	—	6 37	6·36	7·22	6·39	5·79	—	—	6·63	—	—	5·98	7·43

TABLE 45.—*Amino-Acid Nitrogen mg. % (" Unlaked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
22204	4·12	—	—	4·52	5·00	—	—	—	—	—	—	—	—	—	—
25140	5·65	—	—	4·89	—	—	4·11	3·57	—	—	3 68	—	—	4·27	5 78
25142	4·67	—	—	4·36	—	4 75	4 00	—	—	—		—	—	—	—
Av...	4·97	—	—	4 54	5 00	4 75	4 05	3·57	—	—	3·68	—	—	4·27	5·78

" Laked " Filtrates.

Minimum-maximum variation, 5–7·87 mg. N %.

Average, 6·37 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, 2·80–7·00 mg. N %.

Average, 4·57 mg. N %.

Average difference, 1·80 mg. N %.

The differences of the " laked " and " unlaked " figures vary from 10 to 52 % with an average of 28 %.

GROUP D.

Sheep No. 24312, Table 46.

,, No. 29151, Table 47.

,, No. 29468, Table 48.

,, No. 29471, Table 49.

.   No. 29496, Table 50.

,, No. 29503, Table 51.

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TABLE 46.—Sheep 24312.

Date	L/U	12th Nov., 1931	18th Jan., 1931	20th Nov., 1931	5th Feb., 1932	8th Feb., 1932	10th Feb., 1932	16th Feb., 1932	19th Feb., 1932	11th March, 1932	18th May, 1932	20th May, 1932	30th June, 1932	4th July, 1932	28th Sept., 1932	19th Dec., 1932	26th Jan., 1933	31st Jan., 1933
Haemoglobin, gm. per 100 c.c.		11·39	10·99	12·13	15·98	14·72	14·49	12·79	12·42	13 68	15·98	17·51	—	15·19	13·87	15·19	14·08	14·28
Sugar, mg. % (Glucose)	L	47·85	42·37	53·19	—	—	—	48 08	43·48	44·44	48·31	43·67	47·39	40·00	40·98	40·82	57·47	41·66
	U	35·99	37·31	42·55	—	—	—	36·50	36·10	32·26	40·16	32·57	44·44	33·78	37·74	31·15	50·25	26·74
Total-N., gm. N %		2·562	2·520	2 542	3·024	3 001	3·028	2·847	2·751	3 052	3 066	3 234	3 234	3·129	2·968	3·038	2·786	2·590
Non-Protein Nitrogen, mg. %	L	20·27	32·60	30 00	38·96	31 58	30 00	34·28	—	26 08	21·88	20·92	24·50	27·26	21·43	33·16	30·16	34·48
	U	16·21	24·20	23·54	28·04	24 00	23·08	24·80	26·32	19 86	14·71	15·79	11·95	17·75	15·46	23·54	23·54	25·00
Coaguable Nitrogen, gm. N %	L	2·542	2·487	2·512	2·985	2·969	2·998	2 813	2·725	3·026	3·044	3·213	—	3·102	2·947	3·065	2·756	2·556
	U	2·546	2·496	2·518	2·996	2·977	3·005	2·822	—	3·032	3·051	3·218	—	3·111	2·953	3·014	2·763	2·565
Urea, mg. N % / mg. U %	L	5·21 / 10·92	16·62 / 34·90	17·19 / 36·10	16·55 / 34·70	11·27 / 23·60	11·58 / 24·20	16·10 / 33·81	14·63 / 30·70	10·49 / 21·90	4·68 / 9·70	7·33 / 15·40	6·02 / 12·60	9·00 / 18·90	6·43 / 13·44	13·49 / 28·35	6·46 / 13·50	11·20 / 21·42
	U	5·06 / 10·60	15·66 / 32·85	17·34 / 36·40	15·53 / 32·60	12·35 / 32·85	12·25 / 25·70	17·69 / 37·00	—	11·12 / 23·35	4·71 / 9·87	3·44 / 7·20	9·00 / 18·90	7·00 / 14·70	6·32 / 13·23	13·76 / 28·98	6·69 / 14·07	11·87 / 24·99
"Total" Creatinine, mg. N % / mg. TC %	L	— / —	2·48 / 6·70	2·11 / 5·68	2·23 / 6·00	2·33 / 6·26	2·23 / 6·00	2·12 / 5·76	1·98 / 5·34	2·10 / 5·68	2·10 / 5·68	2·01 / 5·40	2·40 / 6·54	2·23 / 6·00	2·23 / 6·00	2·36 / 6·36	2·11 / 5·68	2·48 / ·74
	U	— / —	2·23 / 6·00	1·89 / 5·14	1·78 / 4·80	1·75 / 4·70	1·82 / 4·90	2·41 / 6·54	1·61 / 4·36	1·71 / 4 60	1·41 / 3·80	1·00 / 4·70	1·45 / 3·86	1·82 / 4·90	1·71 / 4·60	1·82 / 4·90	1·56 / 4·16	1·82 / 4·90
Uric acid, mg. N % / mg. UA %	L	·29 / ·86	·33 / 1·00	·26 / ·78	·28 / ·83	·30 / ·91	·24 / ·73	·39 / 1·19	·32 / ·95	·31 / 93	·28 / ·85	·28 / ·84	— / —	·41 / 1·23	·32 / ·97	·38 / 1·14	·30 / ·90	·36 / 1·07
	U	— / —	·17 / ·52	·15 / ·46	·14 / ·43	— / —	— / —	— / —	·18 / ·53	·16 / ·47	·18 / ·53	·14 / ·42	— / —	·18 / ·53	·11 / ·32	·10 / ·31	·10 / ·30	·12 / ·35
Amino Acid, mg. N %	L	7·14	6·09	5·83	7·00	6·36	6·93	6·67	6·73	8 24	7 37	6 45	5·56	6·42	7·37	7·95	7·21	6·48
	U	7·54	5 00	5 18	4·24	5·64	4·86	5·83	5·93	4 76	5·13	3·68	3·87	4·93	4·16	6·36	5·83	4·73
Rest Nitrogen, mg. N %	L	7·63*	7·10	4·61	12·90	11·32	9·02	9 00	—	4 94	7·50	4·85	10·52†	9·20	5·03	7·98	4·08	13·96
	U	3·61*	1·14	0·87	6·35	0·94	4·15	·73	3·97	2 11	3 28	7·53	2·37	3 82	3·16	0·51	9·36	6·46

* Includes "Total" Creatinine-N.   † Includes Uric Acid-N.

History: Born at Onderstepoort, 1.7.29.
12.9.32. Helminthiasis.

Weights: 18.12.31 — 61 lb.
22. 1.32 — 75 lb.
23. 2.32 — 75 lb.
29. 4.32 — 85 lb.
26. 5.32 — 88 lb.
22. 7.32 — 90 lb.
24. 8.32 — 95 lb.
29.12.32 — 98 lb.

TABLE 47.—Sheep 29151.

Date		25th Nov., 1931.	27th Nov., 1931.	8th Feb., 1932.	10th Feb., 1932.	25th Feb., 1932.	29th Feb., 1932.	3rd March, 1932.	26th May, 1932.	2nd June, 1932.	6th June, 1932.	23rd June, 1932.	4th July, 1932.	28th Sept., 1932.	19th Dec., 1932.	26th Jan., 1933.	31st Jan., 1933.
Haemoglobin, gm. per 100 c.c.		9·32	13·68	12·42	11·67	10·35	12·13	11·53	11·67	12·42	12·13	12·79	12·13	12·28	12·94	12·42	13·31
Sugar, mg. % (Glucose)	L	43·48	50·00	—	44·25	48·78	55·87	51·55	56·50	53·76	61·35	60·61	62·11	65·79	47·85	45·66	40·32
	U	40·65	41·66	—	36·36	41·66	44·84	43·48	49·26	43·29	49·26	51·02	51·28	63·29	37·04	38·91	30·96
Total-N., gm. N %		2·401	2·562	2·800	2·730	2·590	2·646	2·674	2·660	2·716	2·688	2·646	2·422	2·555	2·786	2·534	2·576
Non-Protein Nitrogen, mg. %	L	33·32	39·72	28·56	26·20	41·66	28·32	28·04	22·40	20·70	27·14	24·28	20·13	15·87	34·08	23·36	25·00
	U	27·26	33·88	20·84	18·75	33·72	21·82	19·23	12·40	12·10	21·82	12·30	12·50	9·43	27·78	14·42	12·87
Coagulable Nitrogen, gm. N %	L	2·368	2·524	2·771	2·704	2·549	2·618	2·646	2·638	2·695	2·661	2·622	2·402	2·539	2·752	2·511	2·551
	U	2·874	2·530	2·779	2·712	2·556	2·624	2·655	2·648	2·704	2·666	2·634	2·409	2·546	2·759	2·520	2·563
Urea mg. N %	L	16·40	21·48	10·24	8·66	18·61	10·36	6·49	4·06	3·00	7·70	3·65	4·56	4·13	13·66	3·76	5·99
Urea mg. U %	L	34·44	45·00	21·48	18·10	39·06	21·70	13·50	8·50	6·30	16·17	7·60	9·50	8·61	32·97	7·98	12·80
Urea mg. N %	U	16·78	21·87	11·50	8·75	20·51	9·81	7·26	4·33	3·00	7·70	1·85	4·71	4·40	16·70	4·26	5·99
Urea mg. U %	U	35·20	45·80	24·15	18·30	43·05	20·58	15·20	9·03	6·30	16·17	3·80	9·87	9·24	35·07	9·03	12·60
"Total," Creatinine mg. N %	L	2·32	2·32	2·23	2·05	2·23	2·05	2·10	1·90	2·28	2·28	2·49	2·28	2·04	2·59	2·40	2·25
mg. TO %	L	6·26	6·26	6·00	5·54	6·00	5·54	5·68	5·14	6·16	6·16	6·74	6·16	5·50	6·96	6·54	6·12
mg. N %	U	1·78	1·82	1·85	1·71	1·82	1·90	1·67	1·51	2·01	2·11	2·04	1·67	1·56	2·23	1·64	2·11
mg. TC %	U	4·80	4·90	4·96	4·60	4·90	5·14	4·50	4·08	5·40	5·70	5·50	4·50	4·16	6·00	4·80	5·68
Uric Acid mg. N %	L	·35	·30	·30	·22	·29	·32	·23	·21	·21	·18	·20	·31	·25	·25	·25	·31
mg. UA %	L	1·05	·89	·89	·66	·88	·95	·68	·63	·59	·55	·60	·94	·76	·70	·74	·94
mg. N %	U	·19	—	—	—	·17	·17	·07	·12	·09	·10	·09	·20	·08	·09	·11	·10
mg. UA %	U	·57	—	—	—	·50	·52	·22	·36	·26	·31	·27	·59	·25	·27	·34	·30
Amino Acid mg. N %	L	7·00	6·22	6·36	5·96	6·90	7·78	6·83	6·01	6·25	6·48	7·18	5·36	5·74	7·70	6·30	5·74
	U	5·83	5·28	5·83	4·12	5·18	4·83	5·07	4·02	4·52	4·64	4·36	4·12	3·68	5·98	5·26	4·79
Rest Nitrogen, mg. N %	L	7·25	9·40	9·43	9·31	13·63	7·81	12·39	10·22	8·97	10·50	10·76	7·62	3·71	7·88	10·65	10·71
	U	2·68	4·91	1·06	5·17	6·04	2·52	5·16	2·42	2·48	7·27	3·66	1·80	·29	1·78	3·15	−·12

History : Born at Onderstepoort, 6.9.30.
26. 9.30 Helminthiasis.

Weights : 18.12.32 — 49½ ℔.
22. 1.32 — 60 ℔.
22. 2.32 — 59½ ℔.
23. 2.32 — 65 ℔.
29. 4.32 — 65 ℔.
26. 5.32 — 75 ℔.
22. 7.32 — 75 ℔.
24. 8.32 — 77 ℔.
29.12.32 — 74 ℔.

TABLE 48.—Sheep 29468.

		19th Nov., 1931.	25th Nov., 1931.	2nd Feb., 1932.	4th Feb., 1932.	15th Feb., 1932.	18th Feb., 1932.	18th May, 1932.	20th May, 1932.	27th May, 1932.	2nd June, 1932.	6th June, 1932.	5th July, 1932.	7th July, 1932.	28th Sept., 1932.	19th Dec., 1932.	26th Jan., 1933.	31st Jan., 1933.
Haemoglobin, gm. per 100 c.c.		12·42	9·81	11·82	11·24	11·67	10·35	13·31	13·31	10·99	13·31	10·87	11·39	11·14	12·59	11·39	14·08	14·28
Sugar, mg. % (Glucose)	L	40·00	48·54	58·82	47·17	59·88	54·05	54·05	50·25	56·50	59·58	55·55	57·47	58·14	52·91	51·02	45·66	80·65
	U	35·99	42·01	45·45	38·31	52·63	50·50	47·39	37·21	48·31	52·08	45·25	47·39	47·17	49·50	47·62	38·91	63·29
Total-N., gm. N %		2·442	2·352	2·660	2·534	2·618	2·499	2·821	2·709	2·548	2·625	2·583	2·520	2·646	2·660	2·688	2·786	2·618
Non-Protein N., mg. %	L	43·24	36·58	34·48	35·30	28·32	38·96	15·95	18·41	18·63	23·72	20·00	20·00	15·87	28·52	33·32	30·16	32·96
	U	35·30	27·90	27·52	28·98	21·20	28·56	10·07	14·28	12·05	16·39	11·53	13·04	11·03	15·23	25·96	23·54	23·36
Urea mg. N %	L	—	18·05	17·61	20·62	13·60	17·69	3·16	3·00	2·00	8·34	3·00	2·84	t.l.	9·20	17·87	6·46	9·81
Urea mg. U %		—	37·85	36·96	43·30	28·56	37·00	6·60	6·30	4·20	17·50	6·30	5·96	t.l.	19·32	37·59	13·50	20·58
Urea mg. N %	U	—	18·30	20·27	21·22	13·81	17·69	3·16	3·44	3·26	8·34	3·00	2·71	t.l.	5·78	16·40	6·69	11·50
Urea mg. U %		—	38·43	42·50	44·56	29·00	37·10	6·60	7·20	6·80	17·50	6·30	5·75	t.l.	12·18	34·44	14·07	24·15
"Total," Creatinine mg. N %	L	2·40	2·48	2·04	2·23	2·55	2·01	1·86	2·04	2·23	2·11	1·82	2·01	2·23	2·36	2·36	2·11	2·25
"Total," Creatinine mg. TC %		6·54	6·86	5·54	6·00	6·86	5·54	5·02	5·50	5·50	6·00	5·68	4·90	6·00	6·36	6·36	5·68	6·12
"Total," Creatinine mg. N %	U	2·23	1·82	1·56	1·89	2·15	1·89	1·49	1·56	1·75	1·75	1·45	2·11	1·45	2·01	2·01	1·56	1·97
"Total," Creatinine mg. TC %		6·00	4·90	4·16	5·14	5·76	5·14	4·00	4·24	4·70	4·70	3·86	5·68	3·92	5·40	5·40	4·16	5·32
Uric Acid mg. N %	L	·20	·33	·26	·20	·30	·25	·20	·22	·18	·18	·17	·27	·24	·28	·19	·30	·29
Uric Acid mg. UA %		·59	·99	·78	·59	·90	·76	·62	·65	·54	·53	·51	·80	·73	·86	·57	·90	·87
Uric Acid mg. N %	U	—	·12	·11	·09		·16	·17	·14	·10	·08	·10	·21	·14	·08	·10	·10	·12
Uric Acid mg. U A %		—	·36	·32	·26		·49	·50	·42	·31	·25	·29	·62	·42	·24	·31	·30	·37
Amino Acid mg. N	L	5·60	6·22	6·73	5·18	6·67	5·83	6·76	7·00	7·00	6·09	5·18	4·93	5·30	6·14	7·14	7·21	5·46
	U	5·88	5·00	4·24	3·78	5·11	5·00	5·00	4·61	4·98	3·89	3·71	3·33	4·35	3·59	5·98	5·83	4·66
Rest Nitrogen, mg. N %	L	—	9·57	7·77	7·07	5·20	13·18	3·97	7·06	7·22	7·00	9·63	9·95	8·10	8·54	4·60	14·08	15·15
	U	—	2·67	1·33	2·00	0·13	3·82	0·25	4·53	1·96	2·33	3·27	4·64	5·09	3·77	·47	9·36	5·11

History : Born at Bestersput, 19.5.30.
30. 1.31, Haematoporphyrin experiment.

Weights : 18.12.31 — 63 ℔.
22. 1.32 — 72 ℔.
23. 2.32 — 75 ℔.
29. 4.32 — 85 ℔.
26. 5.32 — 85 ℔.
22. 7.32 — 89 ℔.
24. 8.32 — 90 ℔.
29.12.32 — 92 ℔.

TABLE 49.—Sheep 29471.

Date		18th Nov., 1931.	20th Nov., 1931.	5th Feb., 1932.	10th Feb., 1932.	16th Feb., 1932.	19th Feb., 1932.	22nd March, 1932.	12th May, 1932.
Haemoglobin, gm. per 100 c.c.		—	10·97	12·79	13·12	11·53	11·67	12·42	13·50
Sugar, mg. % (Glucose)	L	51·28	47·62	39·84	44·05	50·00	46·30	45·45	47·62
	U	—	45·45	32·26	37·74	42·37	42·37	40·32	35·09
Total-N., gm. N %		—	2·352	2·716	2·807	2 730	2·716	2·772	2·884
Non-Protein N., mg. %	L	26·78	28·08	33 32	24·00	31 74	31·42	28·98	20·00
	U	—	23 54	29·12	15·46	23 16	20·28	20·00	12·93
Coaguable N., gm. N %	L	—	2 323	2 683	2·783	2 698	2·645	2·743	2·864
	U	—	3·328	2·687	2 792	2 707	2·696	2·752	2·872
Urea mg. N %	L	13·04	17·12	12·79	8·22	15·00	—	8 30	4·88
mg. U %	L	27·38	35·95	26·70	17·30	31·50	—	17·43	10·20
mg. N %	U	—	17·19	13·29	8 26	15·40	15·98	8·26	4·88
mg. U %	U	—	36·00	27·80	17·30	32·34	33·50	17·30	10·20
"Total" Creatinine mg. N %	L	2·48	2·86	2·23	2·15	2·33	2·25	2·15	2·04
mg. TC %	L	6·70	6·36	6 00	5·76	6 26	6·12	5·78	5·50
mg. N %	U	—	1·75	1·67	1·67	1·89	1·75	1·78	1·60
mg. TC %	U	—	4·70	4·50	4·70	5·14	4·72	4·80	4·32
Uric Acid mg. N %	L	·25	·08	·25	·21	·32	·33	·24	·25
mg. UA %	L	·76	·26	·76	·62	·97	1·00	·71	·74
mg. N %	U	—	·09	—	—	—	·19	·09	·08
mg. UA %	U	—	·27	—	—	—	·58	·28	·26
Amino Acid, mg. %	L	5 28	5·82	5·49	6·09	6·60	6·36	7·00	6·25
	U	—	5·18	3·78	4·12	5·18	4·67	5·00	4·23
Rest Nitrogen, mg. N %	L	5·73	2·69	14·27	7·33	7·49	—	11·29	6·58
	U	—	·67	10·38	3·08	·69	·23	4·87	2·14

History : Born at Besterspruit, 19.5.30,
18. 5. 32, Bluetongue.
24.12.30, Helminthiasis.

Weights :   18.12.31 — 49 ℔.
22. 1.32 — 66 ℔.
23. 2.32 — 65 ℔.
29. 4.32 — 76 ℔
Discharged, 29.4.32.

TABLE 50.—Sheep 29496.

Date		27th Nov., 1931	1st Dec., 1931	15th Feb., 1932	18th Feb., 1932	22nd Feb., 1932	26th Feb., 1932	2nd March, 1932	13th May, 1932	18th May, 1932	20th May, 1932	23rd June, 1932	30th June, 1932	29th Sept., 1932	19th Dec., 1932	27th Jan., 1933	31st Jan., 1933
Haemoglobin, gm. per 100 c.c.		15·42	11·67	13·12	13·31	13·81	12·59	15·19	16·54	15·19	15·19	16·29	14·08	16·87	14·29	14·49	16·87
Sugar, mg. % (Glucose)	L	47·02	51·55	48·78	54·95	48·08	48·08	47·85	42·92	42·01	44·64	46·87	44·64	45·45	46·73	51·81	42·55
	U	40·65	46·51	38·91	40·65	46·30	39·68	42·01	29·67	30·30	33·11	38·31	35·97	29·41	32·26	34·60	31·95
Total-N., gm. N %		2·226	2·576	2·912	2·807	2·891	2·835	2·982	3·038	3·052	3·066	3·017	3·031	3·080	2·982	2·926	2·940
Non-Protein Nitrogen, mg. %	L	29·12	27·23	24·00	36·82	28·84	35·50	25·00	25·00	18·18	27·40	24·20	23·42	27·02	30·00	25·64	34·08
	U	20·20	20·51	15·63	23·13	20·98	25·86	15·80	12·35	12·73	10·35	13·04	11·86	15·08	21·16	12·77	22·22
Coaguable N., gm. N %	L	2·197	2·549	2·888	2·870	2·862	2·799	2·957	3·013	3·034	3·039	2·992	3·008	3·053	2·952	2·900	2·906
	U	2·206	2·555	2·997	2·884	2·870	2·809	2·966	3·026	3·039	3·056	3·004	3·019	3·065	2·961	2·913	2·918
Urea mg. U % / mg. U %	L	10·83 / 22·7	9·15 / 19·20	7·26 / 15·20	15·12 / 31·71	11·99 / 25·00	— / —	7·47 / 16·68	4·13 / 8·65	3·16 / 6·60	7·33 / 15·33	3·54 / 7·40	4·26 / 8·95	8·17 / 17·22	11·82 / 24·78	8·09 / 17·01	10·88 / 22·89
	U	10·77 / 22·50	9·00 / 18·90	7·20 / 15·12	15·74 / 33·00	10·63 / 22·30	15·66 / 32·80	9·47 / 19·80	4·26 / 8·90	3·00 / 6·30	8·00 / 16·80	1·63 / 3·40	5·89 / 12·30	7·89 / 16·59	11·50 / 24·15	5·66 / 11·76	10·90 / 22·89
"Total" Creatinine mg. N % / mg. TC %	L	2·82 / 6·26	2·54 / 6·86	2·41 / 6·54	2·14 / 5·76	2·14 / 5·76	2·04 / 5·50	1·90 / 5·14	1·90 / 5·14	2·10 / 5·68	2·04 / 5·50	2·41 / 6·54	2·23 / 6·00	2·23 / 6·00	2·29 / 6·00	2·04 / 5·50	2·23 / 0·00
	U	1·82 / 4·90	2·15 / 5·76	1·78 / 4·80	1·57 / 4·24	1·78 / 4·80	1·67 / 4·50	1·51 / 4·08	1·37 / 3·66	1·60 / 4·32	1·38 / 3·72	— / —	1·90 / 5·14	1·67 / 4·50	1·86 / 5·02	1·56 / 4·08	1·82 / 4·90
Uric Acid mg. N % / mg. UA %	L	·27 / ·80	·35 / 1·05	·33 / 1·00	·30 / ·89	·36 / 1·10	·36 / 1·07	·43 / 1·28	·27 / ·81	·23 / ·71	·31 / ·93	·22 / ·67	— / —	·27 / ·82	·36 / 1·07	·41 / 1·23	·39 / 1·19
	U	— / —	·24 / ·73	— / —	·16 / ·47	·17 / ·50	·21 / ·63	·19 / ·58	·16 / ·49	·14 / ·43	·14 / ·43	·08 / ·23	— / —	·09 / ·27	·08 / ·25	·14 / ·42	·17 / ·50
Amino Acid, mg. N %	L	5·28	8·24	7·00	7·22	8·64	7·78	7·78	6·60	7·78	6·48	6·67	5·58	5·30	7·86	7·14	5·93
	U	4·67	6·36	6·09	5·74	5·18	5·18	5·60	4·38	5·43	4·00	4·46	3·33	4·67	6·25	5·83	5·30
Rest Nitrogen, mg. N %	L	10·52	6·95	7·00	12·04	5·71	—	7·42	12·10	4·91	11·24	11·36	11·35*	11·05	6·67	7·96	14·65
	U	2·94	2·76	·56	·08	3·22	3·14	·97	2·34	2·54	3·17	6·67†	·74*	·76	·47	·42	5·03

* Includes Uric Acid-N. † Includes "Total" Creatinine-N.

History: Born at Bestersput, 19.5.30.
30. 1.31. Haematoporphyrin experiment.

Weights: 18.12.31 — 53 lb.
22. 1.32 — 69 lb.
23. 2.32 — 70 lb.
29. 4.32 — 80 lb.
26. 5.32 — 80 lb.
22. 7.32 — 85 lb.
24. 8.32 — 85 lb.
29.12.32 — 90 lb.

TABLE 51.—Sheep 29503.

		25th Nov., 1931.	27th Nov., 1931.	5th Feb., 1932.	8th Feb., 1932.	22nd Feb., 1932.	26th Feb., 1932.	2nd March, 1932.	11th March, 1932.	26th May, 1932.	2nd June, 1932.	6th June, 1932.	7th July, 1932.	8th July, 1932.	28th Sept., 1932.	21st Dec., 1932.	27th Jan., 1933.	1st Feb., 1933.
Date																		
Haemoglobin, gm. per 100 c.c.		10·87	17·51	14·72	14·72	14·72	13·68	14·28	14·95	13·87	15·19	13·31	15·19	14·49	13·68	13·87	13·68	15·42
Sugar, mg. % (Glucose)	L	—	45·25	—	—	50·76	44·44	46·73	48·08	40·82	44·44	52·08	46·95	47·62	45·05	37·88	40·16	38·91
	U	—	42·92	—	—	45·45	36·50	40·32	40·00	33·56	35·71	44·05	40·65	44·64	39·22	30·49	32·68	27·77
Total-N, gm. N %		2·506	2·618	3·010	3·010	3·052	3·080	3·059	2·975	2·982	2·926	2·877	3·024	2·996	2·828	2·758	2·744	2·835
Non-Protein Nitrogen, mg. %	L	31·58	31·90	25·64	27·26	23·24	30·92	18·75	28·16	20·98	20·38	23·42	14·71	14·56	21·14	32·26	25·25	25·32
	U	24·58	26·78	20·42	20·34	16·21	24·28	14·28	17·24	12·00	13·21	13·00	10·75	8·75	12·05	26·64	13·83	16·58
Coagulable Nitrogen, gm. N %	L	2·474	2·586	2·984	2·983	3·029	3·049	3·040	2·947	2·961	2·906	2·854	3·009	2·981	2·807	2·726	2·719	2·810
	U	2·481	2·591	2·990	2·990	3·036	3·056	3·045	2·958	2·970	2·913	2·864	3·013	2·987	2·816	2·732	2·730	2·818
Urea mg. N %	L	15·88	15·53	10·90	10·11	8·32	12·51	5·99	11·82	4·40	3·76	4·92	t.l.	2·84	4·88	18·05	5·25	9·26
mg. U %		33·30	32·55	22·89	21·21	17·85	26·25	12·30	24·80	9·24	7·87	10·30	t.l.	5·90	10·29	38·00	10·92	19·53
mg. N %	U	15·81	15·95	10·90	13·00	8·56	13·29	5·99	10·77	4·00	3·55	4·68	t.l.	4·88	4·88	18·42	5·25	9·00
mg. U %		33·20	33·50	2·89	28·56	17·95	27·80	12·30	22·56	8·40	7·40	9·80	t.l.	10·29	10·29	38·64	10·92	18·90
"Total" Creatinine mg. N %	L	2·05	2·30	2·14	2·32	2·14	2·41	1·71	2·10	2·04	2·10	1·97	2·36	1·82	2·23	2·40	2·29	2·29
mg. TC %		5·54	6·26	5·76	6·26	5·76	6·54	4·60	5·68	5·50	5·68	5·32	6·38	4·90	6·00	6·54	6·16	6·16
mg. N %	U	1·78	1·78	1·49	1·67	1·67	1·90	1·49	1·41	1·52	1·75	1·52	1·55	1·45	2·01	2·10	1·87	2·11
mg. TC %		4·80	4·80	4·00	4·50	4·50	5·14	4·00	3·80	4·08	4·70	4·08	4·16	3·86	5·40	5·68	5·14	5·68
Uric Acid mg. N %	L	·26	·28	·21	·23	·30	·30	·35	·25	·24	·18	·17	·29	·27	·26	·33	·31	·34
mg. UA %		·78	·84	·63	·69	·90	·91	1·05	·75	·71	·55	·52	·86	·80	·78	1·00	·94	1·03
mg. N %	U	·17				·18	·18	·24	·09	·10	·10	·09	·14	·13	·08		·10	·10
mg. UA %		·50				·53	·53	·71	·27	·30	·29	·28	·42	·38	·25		·30	·31
Amino Acid, mg. N %	L	5·60	5·60	5·00	5·83	7·14	7·00	6·36	7·00	5·83	6·42	5·53	5·26	4·24	5·60	5·83	6·36	4·86
	U	4·83	4·52	3·68	4·96	4·24	4·83	5·83	4·40	4·36	3·98	4·58	3·68	3·04	3·68	5·43	4·21	4·16
Rest Nitrogen, mg. N %	L	7·79	7·19	8·39	8·77	5·14	8·70	4·44	6·99	8·47	7·92	10·83	6·80	5·39	8·17	4·65	11·04	8·57
	U	1·99	4·53	4·35	0·11	1·56	4·08	0·83	0·57	2·02	3·83	2·13	5·38	3·95	1·40	−·31	2·40	1·21

History : Born at Bestersput, 19.5.30.
       7. 1.31. Haematoporphyrin experiment.

Weights : 18.12.31 — 50 ℔.
       22. 1.32 — 66 ℔.
       29. 4.32 — 70 ℔.
       26. 5.32 — 72 ℔.
       22. 7.32 — 80 ℔.
       24. 8.32 — 79½ ℔.
       29.12.32 — 78 ℔.

GROUP D. (*Six ewe lambs.*)

TABLE 52.—*Haemoglobin gm. per 100 c.c.*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	11·2	—	—	14·1	13·7	—	16·7	—	15·2	—	13·9	—	—	15·2	14·2
29151	11·5	—	—	11·6	11·5	—	11·7	12·5	12·1	—	12·3	—	—	12·9	12·9
29468	11·1	—	—	11·3	—	—	12·5	12·1	11·3	—	12·6	—	—	11·4	14·2
29471	10·9	—	—	12·3	12·4	—	13·5	—	—	—	—	—	—	—	—
29496	15·4	11·7	—	13·1	15·2	—	15·6	15·2	—	—	16·9	—	—	14·3	15·7
29503	14·2	—	—	14·5	14·6	—	13·9	14·3	14·8	—	13·7	—	—	13·9	14·6
AV...	11·9	—	—	12·8	13·3	—	13·8	12·9	13·3	—	13·9	—	—	13·5	14·3

Minimum-maximum variation gm. per 100 c.c., 9·32–17·51. Average, 13·24.

The following table indicates the distribution:—

gm. per 100 c.c.	No. of determinations.
9–10	2
10–11	7
11–12	13
12–13	16
13–14	18
14–15	14
15–16	11
16–17	4
17–18	2

21 % of the determinations lie between 13 and 14 gm. % and 55 between 12 and 15 gm. %.

TABLE 53.—*Sugar mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	47·8	—	—	45·8	44·4	—	46·0	47·4	40 0	—	41·0	—	—	40·8	44·6
29151	46·7	—	—	49·6	51·6	—	56·5	58·6	62·1	—	65·8	—	—	47·9	43·0
29468	44·3	—	—	55·0	—	—	53·6	57·6	57·8	—	52·9	—	—	51·0	63·2
29471	49·3	—	—	45·1	45·5	—	47·6	—	—	—	—	—	—	—	—
29496	47·6	51·6	—	49·9	47 9	—	43·2	45·8	—	—	45·5	—	—	46·7	47·2
29503	39·9	—	—	47·6	47·4	—	40·8	48·3	47·3	—	45·1	—	—	37·9	39·5
AV...	46·4	—	—	49·2	47·4	—	47·9	52·7	52·0	—	50·0	—	—	44·9	45·5

TABLE 54.—*Sugar mg. % (" Unlaked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
23412	38·6	—	—	36·3	32·36	—	36·4	44·4	33·8	—	37·7	—	—	31·2	38·5
29151	41·2	—	—	40·9	43·5	—	49·3	47·9	57·3	—	63·3	—	—	37·0	38·9
29468	39·0	—	—	46·7	—	—	44·3	48·7	47·3	—	49·5	—	—	47·6	51·1
29471	45·5	—	—	38·7	40·3	—	35·1	—	—	—	—	—	—	—	—
29496	43·6	—	—	41·4	42·0	—	31·0	37·1	—	—	29·4	—	—	32·3	33·3
29503	39·3	—	—	40·9	40·2	—	33·6	39·9	42·6	—	39·2	—	—	30·5	30·2
AV...	40·6	—	—	41·3	39·6	—	38·2	43·9	42·7	—	43·8	—	—	35·7	37·6

" Laked " Filtrates.

Minimum-maximum variation, 34–81 mg. %.
Average, 48·4 mg. %.

" Unlaked " Filtrates.

Minimum-maximum variation, 29–63 mg. %.
Average, 38·0 mg. %.
Average difference, 10·4 mg. %.

The following table indicates the distribution:—

" Laked " Filtrates.			*" Unlaked " Filtrates.*	
mg. %.	*Occurrence.*		*mg. %.*	*Occurrence.*
30–35 … … … …	1		25–30 … … … …	4
35–40 … … … …	3		30–35 … … … …	15
40–45 … … … …	22		35–40 … … … …	21
45–50 … … … …	28		40–45 … … … …	22
50–55 … … … …	16		45–50 … … … …	14
55–60 … … … …	10		50–55 … … … …	6
60–65 … … … …	3		more than 55 …	2
80–85 … … … …	1			

" Laked " Filtrates.

33 % of the determinations lie between 45 and 50 mg. %.
78 % of the determinations lie between 40 and 55 mg. %.

" Unlaked " Filtrates.

25 % of the determinations lie between 40 and 45 mg. %.
70 % of the determinations lie between 30 and 45 mg. %.

The differences between " laked " and " unlaked " vary from 4 to 26 %, with the average 20 %.

Total Nitrogen.

Minimum-maximum variation gm. N % 2·4–3·2.
Average, 2·75 gm. N %.

TABLE 55.—*Non-Protein Nitrogen mg. %* (" Laked ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	**Jan.**
24312	27·6	--	—	33 7	26·1	—	21 4	24·5	27 3	—	21 4	—	—	33·2	**33·8**
29151	36·5	-	—	31·2	28·0	—	22·4	24·0	20·1	—	15·9	—	—	34·1	**24·2**
29468	59·9	-	—	34·3	—	—	17·7	21·9	17·9	—	26 5	—	—	33·3	**31·6**
29471	27 4	--	—	30·1	28 9	—	20·0	--	—	—	—	—	—	—	**—**
29496	29 1	27 2	—	31·3	25·0	—	23·5	23·8	—	—	27 0	—	—	30·0	**29·9**
29503	31·7	-	—	26·8	23 5	--	20 9	21 9	14·6	—	21 1	—	—	32·3	**25·3**
Av...	31 9	—	—	31 2	25 8	—	20 7	23 2	18·8	--	22·4	—	—	32·6	**28·9**

TABLE 56.—*Non-Protein Nitrogen mg. %* (" Unlaked ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	**Jan.**
24312	21·3	—	—	25·4	19·9	—	15·3	11·9	17·8	—	15·5	—	---	23·5	**24·3**
29151	30·6	—	—	23·8	19·2	—	12·4	15·3	12·5	—	9·4	—	—	27·8	**13·7**
29468	31·5	—	—	26·6	—	—	12·1	13·9	12·0	—	15·2	--	—	25·9	**23·5**
29471	23·5	—	—	22·0	20·0	—	12·9	—	—	—	—	—	—	—	**—**
29496	20·4	—	—	21·4	15·8	—	12·6	12·5	—	—	15 1	—	—	21·1	**17·5**
29503	25·7	—	—	20·3	15·8	—	12·0	13·1	10·8	—	12·1	—	—	26·6	**15·2**
Av...	25·1	—	—	23·5	17·3	—	13·8	13·7	13·0	—	13·5	—	—	25·0	**18·8**

" *Laked* " *Filtrates.*

Minimum-maximum variation, 14·6–43·2 mg. N %.
Average, 26 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, 8·6–35·3 mg. N %.
Average, 18 mg. N %.
Average difference, 8 mg. N %.

The following table indicates the distribution:—

" *Laked* " *Filtrates.*		" *Unlaked* " *Filtrates.*	
mg. %.	*Occurrence.*	*mg. %.*	*Occurrence.*
below 15	2	below 15	27
20–20	7	15–20	14
20–25	22	20–25	23
25–30	26	above 25	16
30–35	24		

" *Laked* " *Filtrates.*

32 % of the determinations lie between 25 and 30 mg. N %.
62 % of the determinations lie between 25 and 35 mg. N %.

" *Unlaked* " *Filtrates.*

32 % of the determinations lie below 15 mg. N %.
The percentage differences of the " laked " and " unlaked " figures vary between 16 % to 48 % with an average of 31 %.

TABLE 57.—*Urea Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	13·0	—	—	13·9	10·5	—	6·0	6·0	9·0	—	6·4	—	—	13·5	8·8
29151	18·9	—	—	11·9	6·5	—	4·1	4·6	4·6	—	4·1	—	—	15·7	4·9
29468	18·1	—	—	17·2	—	—	2·7	5·7	1·4	—	9·2	—	—	17·9	8·1
29471	15·1	—	—	12·0	8·3	—	4·9	—	—	—	—	—	—	—	—
29496	10·0	—	—	11·5	—	—	4·8	3·9	—	—	8·2	—	—	11·8	9·5
29503	15·7	—	—	10·5	8·9	—	4·4	4·3	1·4	—	4·9	—	—	18·1	7·3
Av...	14·5	—	—	12·9	8 4	—	4·7	4·8	3·2	—	6·6	—	—	15·4	7·7

" *Laked* " *Filtrates.*

Minimum-maximum variation, below 1·0–21·48 mg. N %.
Average, 9·28 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, below 1·0–21·87 mg. N %
Average, 9·28 mg N %.

The following table indicates the distribution of the " laked ":—

Below 4	12
4– 5	10
5– 6	4
6– 7	5
7– 8	5
8– 9	6
9–10	5
10–11	6
above 11	31

20 % of the determinations lie between 6 and 9 mg. N %.

TABLE 58.—" *Total Creatinine Nitrogen mg. %* (" *Laked* ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	2·3	—	—	2·2	2·1	—	2·1	2·4	2·2	—	2·2	—	—	2·4	2·3
29151	2·3	—	—	2·1	2·1	—	1·9	2·4	2·3	—	2·0	—	—	2·6	2·3
29468	2·4	—	—	2·2	—	—	2·0	1·9	2·1	—	2·4	—	—	2·4	2·2
29471	2·4	—	—	2·2	2·2	—	2·0	—	—	—	—	—	—	—	—
29496	2·3	2·5	—	2·2	1·9	—	2·0	2·3	—	—	2·2	—	—	2·3	2·3
29503	2·2	—	—	2·3	1·9	—	2·0	2·0	2·1	—	2·2	—	—	2·4	2·3
Av...	2·3	—	—	2 2	2·0	—	2·0	2·2	2·2	—	2·2	—	—	2·4	2·3

TABLE 59.—" *Total* " *Creatinine Nitrogen mg. %* (" *Unlaked* ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	2·1	—	—	1·9	1 7	—	1·2	1·5	1 8	—	1·7	—	—	1·8	1·7
29151	1·8	—	—	1 8	1·7	—	1·5	2·1	1·7	—	1·6	—	—	2·2	1·9
29468	2·0	—	—	1 9	—	—	1·6	1·6	1·8	—	2·0	—	—	2·0	1·8
29471	1·8	—	—	1·7	1·8	—	1·6	—	—	—	—	—	—	—	—
29496	2·0	—	—	1·7	1·5	—	1·5	1·9	—	—	1 7	—	—	1·9	1·7
29503	1·8	—	—	1·7	1·5	—	1·5	1·6	1·5	—	2·0	—	—	2·1	2·0
Av...	1·9	—	—	1·8	1 6	—	1 5	1·8	1·7	—	2·0	—	—	2·0	1·8

" *Laked* " *Filtrates.*

Minimum-maximum variation, 1·71–2·55 mg. N %.

Average, 2·22 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, 1·0–2·41 mg. N %.

Average, 1·75 mg. N %.

Average difference, 0·47 mg. N %.

The differences between " laked " and " unlaked " " T ".C.N. vary from 5 % to 34 %, with an average of 21 %.

TABLE 60.—*Uric-Acid Nitrogen mg. %* (" *Laked* ").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	·29	—	—	·31	·31	—	·28	—	·41	—	·32	—	—	·38	·33
28151	·32	—	—	·28	·23	—	·21	·31	—	—	·25	—	—	·25	28
29468	·25	—	—	·27	—	—	·20	·18	·25	—	·28	—	—	·19	·30
29471	·16	—	—	·28	·24	—	·25	—	—	—	—	—	—	—	—
29496	·27	·35	—	·34	·43	—	·27	·22	—	—	·27	—	—	·36	·40
29503	·27	—	—	·26	·30	—	·24	·18	28	—	·26	—	—	·33	·32
Av...	·26	—	—	·28	·30	—	24	17	30	—	28	—	—	·30	·33

" *Laked* " *Filtrates.*

Minimum-maximum variation, below ·10–·52 mg. N %.

Average, 0·36 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, below ·10–·24 mg. N %.

TABLE 61.—*Amino-Acid Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	6·4	—	—	6·7	8·2	—	6·9	5·6	6·4	—	7·4	—	—	7·9	6·8
29151	6·6	—	—	6·8	6·8	—	6·0	6·6	5·4	—	5·7	—	—	7·7	6·0
29468	5·9	—	—	6·1	—	—	6·6	8·4	5·1	—	6·1	—	—	7·1	6·3
29471	5·6	—	—	6·1	7·0	—	6·3	—	—	—	—	—	—	—	—
29496	6·8	8·2	—	7·7	7·8	—	7·9	6·1	—	—	5·3	—	—	7·9	6·5
29503	5·6	—	—	6·2	6·7	—	5·8	5·9	4·9	—	5·6	—	—	5·8	5·6
Av...	6·2	—	—	6·2	7·2	—	6·6	6·1	5·3	—	6·0	—	—	7·3	6·3

TABLE 62.—*Amino-Acid Nitrogen mg. % (" Unlaked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
24312	5·9	—	—	5·3	4·8	—	4·4	3·9	4·9	—	4·2	—	—	6·4	5 3
29151	5·6	—	—	4·9	5·1	—	4·0	4·5	4·1	—	3·7	—	—	5·9	5 0
29468	5·2	—	—	4·5	—	—	4·9	3·8	3·8	—	3·6	—	—	5·9	5 2
29471	5·2	—	—	4·4	5·0	—	4·2	—	—	—	—	—	—	—	—
29496	4·7	6·4	—	5·6	5·6	—	4·6	3·9	—	—	4·7	—	—	6·3	5·6
29503	4·7	—	—	4·4	5·1	—	4·4	4·3	3·4	—	3 7	—	—	5·4	4 2
Av...	5·3	—	—	4·9	5·1	—	4·5	4·1	3 9	—	3·9	—	—	6 0	5·1

" Laked " Filtrates.

Minimum-maximum variation, 4·86–7·14 mg. N %.
Average, 6·39 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, 3·04–7·54* mg. N %.
Average, 4·80 mg. N %.
Average difference, 1·59 mg. N %.

The differences of the " laked " and " unlaked " figures vary from 0* to 43 %, with the average of 28·5 %.

GROUP E.

Sheep No. 28437, Table 63.

,,   No. 31662, Table 64.

,,   No. 31742, Table 65.

,,   No. 31905, Table 66.

,,   No. 32176, Table 67.

* Vide table 46, 12th November, 1931.

TABLE 63.—Sheep 28437.

Date		29th Oct., 1931.	3rd Nov., 1931.	2nd Dec., 1931.	3rd Dec., 1931.	4th March, 1932.	17th March, 1932.	31st March, 1932.	29th April, 1932.	3rd May, 1932.	6th May, 1932.	30th June, 1932.	5th July, 1932.	12th July, 1932.	30th Sept., 1932.	21st Dec., 1932.	27th Jan., 1933.	1st Feb., 1933.
Haemoglobin, gm., per 100 c.c.		12·28	13·77	11·67	11·96	11·67	14·08	11·96	17·18	14·95	13·87	14·28	15·98	14·28	10·14	12·28	11·24	13·31
Sugar, mg. %		36·76	38·61	52·63	52·08	45·25	39·65	41·15	84·03	51·55	52·63	52·63	43·10	49·50	44·25	43·10	48·54	38·91
(Glucose)		33·0	31·65	43·10	41·66	42·65	35·09	36·10	76·34	47·74	42·37	45·05	37·45	37·88	37·17	42·01	40·98	27·77
Total N., gm. N %		2·422	2·625	2·590	2·534	2·702	2·884	2·738	3·122	3·045	2·828	2·968	3·080	2·940	2·688	2·695	2·492	2·835
Non-Protein Nitrogen. mg. %	L	—	15·95	28·98	24·20	24·00	26·20	24·40	17·97	26·42	21·06	22·72	20·42	18·69	17·44	27·66	16·86	25·32
	U	12·42	10·53	23·08	17·44	—	16·13	15·31	11·77	19·23	14·16	13·39	13·39	11·53	11·45	21·58	10·91	15·68
Coaguable Nitrogen. gm. N %	L	—	2·609	2·561	2·510	2·678	2·858	2·734	3·104	3·019	2·807	2·945	3·060	2·921	2·671	2·667	2·475	2·810
	U	2·410	2·614	2·567	2·517	—	2·868	2·743	3·110	3·026	2·814	2·955	3·067	2·928	2·677	2·673	2·481	2·818
Urea. mg. N %	L	4·44	4·00	16·47	8·90	8·13	8·34	9·20	5·15	7·70	6·40	6·26	3·00	2·71	4·88	13·76	3·00	8·14
mg. U %	U	9·30	8·40	34·50	18·69	17·01	17·50	19·32	10·71	16·17	13·44	13·10	6·30	5·67	10·29	28·98	6·30	17·01
mg. N %	L	5·06	4·08	17·19	9·25	—	8·52	9·20	5·55	7·26	6·80	5·25	1·25	2·57	4·71	14·10	3·00	6·94
mg. U %	U	10·60	8·40	36·00	19·40	—	17·80	19·32	11·60	15·20	13·23	11·00	2·60	5·35	9·87	29·61	6·30	14·49
mg. N %	L	2·01	2·04	2·41	2·32	2·01	2·15	2·05	2·01	1·67	1·90	2·35	2·14	2·23	2·11	1·82	1·71	2·29
mg. TC %	U	5·54	3·54	6·54	6·25	5·40	4·80	5·54	5·40	4·50	5·14	6·36	5·78	6·00	5·68	4·90	4·60	6·16
"Total", Creatinine. mg. N %	L	1·82	1·86	1·85	1·82	1·52	1·34	1·72	1·41	1·25	1·64	1·75	1·67	1·78	1·67	1·54	1·44	2·11
mg. TC %	U	4·90	5·02	4·96	4·90	4·08	3·60	4·65	3·80	3·36	4·40	4·70	4·50	4·80	4·50	4·16	3·86	5·68
mg. N %	L	—	·26	·32	·27	·16	·30	·20	·28	·26	·22	—	·28	·24	·19	·39	·25	·34
mg. UA %	U	—	·77	·95	·82	·48	·91	·59	·83	·77	·65	—	·84	·73	·56	1·18	·75	1·03
Uric acid. mg. N %	L	—	—	—	·15	—	·15	·11	·19	·13	·14	—	·20	·10	·08	·16	·14	·10
mg. UA %	UU	—	—	—	·46	—	·44	·33	·57	·38	·42	—	·59	·29	·24	·48	·42	·30
Amino acid, mg. N %	L	7·11	7·18	5·83	6·09	7·00	5·83	5·79	7·14	7·37	6·09	5·38	5·83	5·36	6·54	6·80	5·93	4·86
	U	5·38	5·38	5·18	5·18	—	4·67	5·18	4·86	4·75	5·18	3·85	4·35	3·50	4·35	5·79	5·88	4·16
Rest Nitrogen, mg. N %	L	—	—	3·95	6·62	6·70	9·68	7·16	3·39	9·42	6·45	5·38	9·17	8·15	3·72	3·89	5·97	9·69
	U	·16	—	—	1·04	—	1·45	·90	·24	5·84	·90	3·85	5·92	3·58	·64	1·01	·95	3·27

History : 24.5.30 — Bluetongue.
27.6.30 — Helminthiasis.
25.9.30 — Feeding experiment.

Weights : 18.12.31 — 117 ℔.
22. 1.32 — 136 ℔.
23. 2.32 — 118½ ℔.
29. 4.32 — 128 ℔.
26. 5.32 — 130 ℔.
22. 7.32 — 135 ℔.
24. 8.32 — 137 ℔.
29.12.32 — 135 ℔.

TABLE 64.—Sheep 31662.

Date.		28th Oct., 1931.	30th Oct., 1931.	26th Nov., 1931.	3rd Dec., 1931.	25th Feb., 1931.	29th Feb., 1932.	3rd March, 1932.	29th April, 1932.	3rd May, 1932.	6th May, 1932.
Haemoglobin, gm., per 100 c.c.		12·42	12·79	12·94	12·28	12·13	14·49	13·31	15·42	15·19	14·28
Sugar, mg. %. (Glucose)	L	34·48	45·05	46·73	45·87	51·55	51·55	46·08	53·19	48·08	51·02
	U	33 33	48 54	40·32	36·00	33·67	37·07	40·00	41·84	38·31	40·82
Total N., gm. N %		2·681	2·660	2·786	2·744	2·856	3 080	2·926	2·954	3·150	3·024
Non-Protein-N., mg. %.	L	26·52	—	34·48	26·32	33·16	30·92	34·68	25 10	27·02	24·90
	U	20 00	—	26·52	20·56	22·98	22 30	26·20	16 13	18·99	17·24
Coagulable N., gm. N %.	L	2·654	—	2 752	2·718	2·823	3 050	2·891	2 929	3·123	2·999
	U	2·661	—	2·759	2·724	2·833	3·058	2·900	2·988	3·131	3·007
Urea. mg. N % / mg. U %	L	9·20 / 19·32	6 27 / 13 18	14·33 / 33·03	11·25 / 23 60	12·03 / 25·20	9·58 / 20·00	10·90 / 22·89	10·77 / 22·00	5·66 / 11·86	7·33 / 15 40
	U	9·82 / 20 60	5·94 / 12·40	14·63 / 33·70	11·00 / 23·10	12·42 / 26·04	9·42 / 19·74	10 17 / 21·30	10·97 / 22·95	5·49 / 11·40	7·33 / 15·33
"Total"-Creatinine. mg. N % / mg. TC %	L	2·66 / 7·20	2 32 / 6 26	2 25 / 6·12	2·80 / 7·58	2·18 / 5 86	2·23 / 6·00	2·10 / 5·68	2 16 / 5·84	1·82 / 4·90	2·66 / 7·20
	U	2·23 / 6·00	1·78 / 4·80	1·82 / 4·90	2·09 / 5·64	1·75 / 4·70	1·90 / 5·14	1·55 / 4·16	1·44 / 3·86	1·29 / 3·48	·93 / 2·50
Uric acid. mg. N % / mg. UA %	L	— / —	·38 / 1·14	·40 / 1·19	·38 / 1·14	·34 / 1·02	·36 / 1·08	·23 / ·70	·32 / ·96	·31 / ·91	·29 / ·87
	U	— / —	— / —	·19 / ·57	— / —	·14 / ·42	·16 / ·47	·09 / ·27	·18 / ·55	·13 / ·38	·14 / ·41
Amino acid, mg. N %.	L	7·49	6·67	6·36	6 67	8·24	8·48	8·24	5·98	8·24	8·24
	U	6 36	4 79	4·79	5·00	5·74	5·18	5·00	4·00	4·88	5·83
Rest Nitrogen, mg. N %.	L	7·17*	—	11·14	5 22	10 73	10 27	13·21	5·87	10·99	6·27
	U	1 59*	—	5 09	2·17	2 98	5 64	8·79	·46	7·70	3·01

* Includes Uric Acid N.

History : 10. 6.31 — Bluetongue.

Weights : 18.12.31 — 78 lb.
22. 1.32 — 98¾ lb.
23. 2.32 — 95 lb.
29. 4.32 — 97 lb.
29. 4.32 — Discharged

TABLE 65.—Sheep 31742.

Date.		29th Oct., 1931.	3rd Nov., 1931.	2nd Dec., 1931.	10th Dec., 1931.	4th March, 1932.	22nd March, 1932.	31st March, 1932.	12th May, 1932.	1st June, 1932.	8th July, 1932.	12th July, 1932.	30th Sept., 1932.	22nd Dec., 1933.	25th Jan., 1933.	1st Feb., 1933.
Haemoglobin, gm. per 100 c.c.		13 12	16 29	13 31	15 19	13 50	15 42	14 28	14 72	15 19	17 18	15 42	12 59	17 51	17 51	12 13
Sugar, mg. % (Glucose)	L	40 82	—	43 48	37 17	51 28	41 84	39 84	39 06	41 84	48 08	43 48	51 28	50 00	50 76	54 64
	U	32 79	—	27 47	33 34	40 98	31 25	29 15	29 33	33 00	37 88	29 41	39 22	32 26	36 90	35 97
Total N, gm. N %		2 926	2 968	2 968	2 814	2 940	2 171	3 094	2 996	3 130	2 982	3 108	2 919	3 080	2 954	2 772
Non-Protein Nitrogen, mg. %.	L	—	20 54	28 98	29 42	—	23 62	26 32	25 64	26 78	17 85	18 75	20 70	37 50	24 50	23 42
	U	12 50	12 50	23 72	21 72	—	15 00	13 10	14 71	15 29	9 43	10 10	16 66	27 02	15 39	14 42
Coagulable Nitrogen, gm. N %.	L	—	2 947	2 939	2 785	—	3 147	3 068	2 970	3 109	2 964	3 089	2 898	3 053	2 930	2 749
	U	2 913	2 955	2 944	2 792	—	3 156	3 081	2 981	3 121	2 974	3 098	2 902	3 063	2 939	2 758
Urea. mg. N % / mg. U %	L	5 45 / 11 40	7 16 / 15 00	11 50 / 24 15	13 14 / 27 60	6 55 / 13 70	5 60 / 11 76	6 40 / 13 44	5 55 / 11 60	6 81 / 14 30	2 78 / 5 80	3 00 / 6 30	6 07 / 12 81	19 62 / 41 16	5 10 / 10 71	5 54 / 11 55
	U	5 06 / 10 60	7 70 / 16 17	10 70 / 22 47	13 29 / 27 90	7 00 / 14 00	4 85 / 10 20	6 55 / 13 70	5 71 / 11 97	6 81 / 14 28	t 1	3 66 / 7 60	3 53 / 11 55	19 84 / 41 58	5 21 / 10 92	8 01 / 16 80
"Total," creatinine. mg. N % / mg. TC %	L	1 97 / 5 32	2 23 / 6 00	2 36 / 6 40	2 81 / 7 58	1 90 / 5 14	1 97 / 5 32	2 23 / 6 05	1 97 / 5 32	2 35 / 6 36	2 11 / 5 68	2 17 / 5 68	2 01 / 6 40	2 23 / 6 36	2 23 / 6 00	2 44 / 6 54
	U	1 90 / 5 14	1 82 / 4 90	2 01 / 5 40	2 32 / 6 26	1 57 / 4 24	1 45 / 3 88	1 78 / 4 80	1 60 / 4 32	1 78 / 4 80	1 38 / 3 72	1 75 / 4 70	1 56 / 4 16	2 04 / 5 50	1 52 / 4 16	1 75 / 4 70
Uric acid. mg. N % / mg. UA %	L	— / —	30 / 91	38 / 1 14	34 / 102	25 / 74	24 / 73	24 / 73	24 / 72	27 / 82	33 / 1 00	30 / 90	22 / 67	44 / 1 33	36 / 1 10	39 / 1 18
	U	— / —	— / —	— / —	10 / 31	11 / 33	08 / 25	13 / 40	07 / 22	17 / 51	12 / 35	10 / 30	09 / 26	14 / 42	15 / 46	11 / 32
Amino acid, mg. N %.	L	5 71	6 36	6 19	6 80	7 00	6 80	7 95	6 33	4 65	4 79	5 49	4 45	8 54	7 69	5 64
	U	4 52	3 50	4 67	5 38	—	4 83	5 83	4 88	2 80	3 98	3 33	4 45	5 42	5 88	5 15
Rest Nitrogen, mg. N %.	L	1 02*	4 49	8 55	6 33	—	9 01	9 50	11 55	12 70	7 84	7 85	4 95	5 54	9 12	9 41
	U	—	48	6 34	63	—	2 79	26	3 95	2 83	3 85	1 26	4 94	— 1 42	3 13	— 0 6

* Includes Uric Acid N.

History : 3. 6. 32 — Bluetongue.

Weights : 18.12.31 — 85 lb.
22. 1.32 — 100 lb.
23. 2.32 — 92½ lb.
29. 4.32 — 100 lb.
26. 5.32 — 99 lb.
22. 7.32 — 101 lb.
24. 8.32 — 105 lb.
29.12.32 — 108 lb.

TABLE 66.—Sheep 31905.

Date.		28th Oct., 1931.	30th Oct., 1931.	26th Nov., 1931.	3rd Dec., 1931.	25th Feb., 1932.	2nd March, 1932.	26th March, 1932.	29th April, 1932.	9th May, 1932.
Haemoglobin, gm., per 100 c.c.		10·99	12·94	12·79	11·67	12·13	13·31	13·12	14·28	14·95
Sugar, mg. %	L	52·08	55·55	44·84	51·02	54·64	51·02	47·62	64·52	46·73
(Glucose)	U	46·99	47·62	41·84	47·62	41·66	44·44	37·45	53·48	38·46
Total N, gm. N %.		2·590	2·695	2·744	2·744	2·891	2·954	3·080	3·052	3·122
Non-Protein N., mg. %.	L	21·42	14·28	29·26	27·90	33·16	21·43	29·12	24·80	18·52
	U	14·71	—	21·42	23·06	26·08	16·66	20·83	16·66	11·32
Coagulable Nitrogen, gm. N %.	L	2·569	2·681	2·715	2·716	2·858	2·933	3·051	3·027	3·103
	U	2·576	—	2·723	2·721	2·865	2·937	3·059	3·035	3·111
Urea mg. N %	L	5·67	2·77	10·11	10·17	8·26	8·52	9·20	8·85	5·15
mg. U %	U	11·80	5·70	21·21	21·30	17·30	17·85	19·32	18·50	10·81
mg. N %	L	6·82	3·55	10·05	10·24	10·30	9·58	10·63	8·90	4·97
mg. U %	U	14·30	7·40	21·00	21·50	21·83	20·00	21·70	18·69	10·35
mg. N %	L	2·41	2·32	2·32	2·66	2·14	1·90	2·01	2·04	1·90
mg. TO %		6·54	6·26	6·26	7·20	5·76	5·11	5·40	5·50	5·14
"Total" creatinine. mg. N %	U	2·14	1·78	1·82	2·04	1·49	1·49	1·75	1·60	1·44
mg. TC %		5·76	4·80	4·90	5·50	4·00	4·00	4·70	4·32	3·86
mg. N %	L	—	·23	·32	·31	·29	·35	·34	·32	·23
mg. UA %		—	·70	·97	·94	·86	1·05	1·02	·97	·70
Uric acid mg. N %	U	—	—	·15		·15	·09	·17	·20	·11
mg. UA %		—	—	·45		·45	·27	·52	·59	·32
Amino acid, mg. N %.	L	6·67	6·36	5·71	6·67	7·37	7·07	7·78	6·03	6·86
	U	5·83	5·22	4·67	5·18	4·52	5·71	4·83	4·38	5·00
Rest Nitrogen, mg. N %.	L	6·67*	2·60	10·80	8·09	5·10	3·59	9·79	7·56	4·88
	U	·08*	—	4·88	5·60	—	·21	3·45	1·58	·20

* Includes uric acid N.

History : 16. 5.31 — Bluetongue.
27. 8.31 — Black quarter.

Weights : 18.12.31 — 100 lb.
22. 1.32 — 121 lb.
23. 2.32 — 118 lb.
29. 4.32 — 131 lb.
29. 4.32 — Discharged.

TABLE 67.—Sheep 32176.

Date.		28th Oct., 1931.	30th Oct., 1931.	26th Nov., 1931.	3rd Dec., 1931.	4th March, 1932.	22nd March, 1932.	31st March, 1932.	29th April, 1932.	9th May, 1932.	1st June, 1932.	23rd June, 1932.	4th July, 1932.	30th Sept., 1932.	22nd Dec., 1932.	25th Jan., 1933.	1st Feb., 1933.
Haemoglobin, gm., per 100 c.c......		10·47	11 67	11·14	12 42	12 94	14 28	13 31	14 08	13 87	12·28	14 95	14·72	11 67	14 72	14·28	13·50
Sugar, mg. %............	L	—	58·14	50 00	48 31	52 08	43 48	44 25	56 82	44 84	51 28	48 78	53 19	42·74	43 48	46·08	54·35
(Glucose)...............	U	—	51 55	38 31	41 66	43 86	36 90	36 97	46 87	38·17	46 51	46 08	44 64	33·67	32·26	36·76	35·97
Total N., gm. N %.........		2 464	2·506	2·604	2 653	2·772	3 094	2 898	2 954	3 038	2 639	2 996	2 905	2 786	2·856	2·776	2 730
Non-Protein-N., mg. %.	L	20 92	—	30 46	28 84	27 26	29 42	26 52	30 46	23 82	21 28	24·80	19 48	17 85	27 90	21·58	24·20
	U	15 39	—	24 00	21 28	16 66	19 11	15 23	22 90	14 63	12 87	13·70	10 91	12·60	21·82	15 00	15·15
Coaguable-N., gm. N %.	L	2 443	—	2 574	2 625	2 745	3 064	2 871	2 924	3 014	2·618	2 971	2 886	2 768	2 828	2·754	2·706
	U	2 449	—	2·580	2 632	2 756	3 074	2·883	2·931	3 023	2 626	2·982	2 894	2·773	2·824	2·761	2 715
Urea. mg. N %......	L	5 71	4 95	9 75	10 70	8 75	10 36	8 61	15 33	7·85	4 00	4 50	1·69	4 62	14·65	6·09	7·03
mg. U %......	L	11 97	10 30	20 40	22 47	18 30	21 70	18 06	32 60	16·40	8·40	9 45	3·40	9·66	30·76	12·81	14·70
mg. N %......	U	6 41	5 67	9 75	10 77	8 34	10 77	8 61	16 65	5 77	4 00	3 08	2·22	3·85	14 81	5·99	7·48
mg. U %......	U	13 44	11·80	20 45	22 50	17 50	22 50	18 06	34 90	12 00	8·40	6 93	4·70	6·93	31 08	12·60	15·75
"Total" creatinine. mg. N %......	L	2 48	2 05	2 32	2 66	1 97	2 14	2 23	1 90	1 86	2 01	2 28	2·01	2 01	2 41	2·59	2·36
mg. TC %......	L	6 70	5·54	6 26	7 20	5 32	5 76	6 00	5 14	5 02	5 40	6 16	5·40	5·40	6·54	6·96	6·36
mg. N %......	U	2 23	1 78	1 82	2 01	1 78	1 67	1 97	1 60	1 45	1 75	1 90	1·60	1 75	2 01	1·97	1·86
mg. TC %......	U	6 00	4 80	4 90	5 42	4 80	4 50	5 32	4 32	3 92	4·70	5·14	4·32	4·70	5 40	5·32	5·02
Uric Acid. mg. N %......	L	—	·28	·34	·28	·21	·23	·23	·30	·24	·26	·32	·34	·30	·34	·25	·26
mg. UA %......	L	—	·85	1 03	·84	·64	·68	·68	·91	·73	·78	·65	1·03	·91	1·03	·76	·78
mg. N %......	U	—	—	·17	—	·11	·08	·12	·19	·12	·17	·08	·20	·08	·15	·17	·11
mg. UA %......	U	—	—	·50	—	·34	·23	·35	·58	·37	·52	·23	·60	·24	·44	·50	·33
Amino acid, mg. N %.	L	6 67	6 42	7 00	5 96	7 18	7·00	8.14	6 60	6 09	4 19	6 36	5 76	5·83	6·19	5·93	5·46
	U	5 71	4 66	5 38	5 38	5 18	4 12	5 97	4 52	4·12	3·26	4 12	4·81	4 97	5·38	5·26	4·67
Rest Nitrogen, mg. N %.	L	6 06*	—	13 05	11 24	9 15	9 69	7 31	6 13	7·78	1·82	11 34	9·68	5 10	4·31	6·72	2·09
	U	1 04*	—	6 88	3 12	1 25	2 47	1 45	00	·17	2·69	4 52	2·08	2·45	- 0·53	1·61	1 03

* Includes uric acid N.

History : 26. 5.31 — Bluetongue.
Weights : 18.12.31 — 92¾ lb.
22. 1.31 — 112 lb.
22. 2.32 — 104 lb.
29. 4.32 — 115 lb.
26. 5.32 — 117 lb.
22. 7.32 — 120 lb.
24. 8.32 — 123 lb.
29.12.32 — 118 lb.

GROUP E (*six-tooth wethers*).

TABLE 68.—*Haemoglobin gm. per 100 c.c.*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	13·0	11·8	—	—	12·6	17·2	14·4	14·3	15·1	—	10·1	—	—	12·3	12·3
31662	12·7	12·3	—	13·3	13·3	15·4	14·7	—	—	—	—	—	—	17·5	—
31742	14·7	14·3	—	—	14·4	—	14·7	15·2	16·3	—	12·6	—	—	17·5	14·8
31905	12·2	11·7	—	12·1	13·2	14·3	14·9	—	—	—	—	—	—	—	—
32176	11·1	12·4	—	—	13·5	14·1	13·9	13·6	14·7	—	11·7	—	—	14·7	13·9
Av...	12·6	12·6	—	12·9	13·4	15·2	14·6	14·2	15·6	—	11·5	—	—	14·8	13·7

Minimum-maximum variation, 10·1–17·6 gm. per 100 c.c.
Average, 12·18 gm. per 100 c.c.

The following table indicates the distribution:—

gm. per 100 c.c.	Occurrence.
10–11	3
11–12	8
12–13	13
13–14	12
14–15	16
15–16	6
16–17	1
17–18	5

25 % of the determinations lie between 14 and 15 gm. per 100 c.c. and 63 % between 12 and 15 gm. per 100 c.c.

TABLE 69.—*Sugar mg. % (" Laked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	37·7	52·4	—	—	42·0	84·0	52·1	52·6	46·3	—	44·3	—	—	43·1	43·7
31662	46·7	45·9	—	51 6	46·1	53·2	49·6	—	—	—	—	—	—	50·0	—
31742	39·4	40·3	—	—	44·3	—	41·7	41·8	45·8	—	51·3	—	—	50·0	52·7
31905	50·2	51·0	—	54·6	49·3	64·5	46·7	—	—	—	—	—	—	—	—
32176	54·1	48·3	—	—	46·6	56·8	44·8	50·0	53·2	—	42·7	—	—	43·5	50·2
Av...	45·4	47·3	—	52·9	46·1	64·5	48·0	48·6	47·5	—	46·1	—	—	45·5	48·9

TABLE 70.—*Sugar mg. % (" Unlaked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	32·4	42·4	—	—	37·9	76·3	45·1	45·1	37·7	—	37·2	—	—	42·0	34·4
31663	40·7	36·0	—	35·4	40·0	41·8	38·6	—	—	—	—	—	—	—	—
31742	35·2	31·4	—	—	33·8	—	29·3	33·0	33·7	—	39·2	—	—	32·3	36·4
31905	45·5	47·6	—	41·7	40·9	53·5	38·5	—	—	—	—	—	—	—	—
32176	47·8	41·7	—	—	38 9	46·9	38·2	46·3	44·6	—	33·7	—	—	32·3	36·4
Av...	40·9	39·0	—	37·5	37·8	54·6	39·3	42·7	37·5	—	36·7	—	—	35·5	35·7

" Laked " Filtrates.

Minimum-maximum variation, 36–84 mgm. %.
Average, 48·4 mg. %.

" Unlaked " Filtrates.

Minimum-maximum variation, 27–76 mg. %.
Average, 39·6 mg. %.
Average difference, 8·8 mg. %.

The following table indicates the distribution:—

mg. %.	Occurrence.	mg. %.	Occurrence.
30–35	1	25–30	6
35–40	9	30–35	10
40–45	14	35–40	21
45–50	14	40–45	17
50–55	22	45–50	10
55–60	3	50–55	2
60–65	1	more than 65 ...	1
more than 65 ...	1		

" Laked " Filtrates.

33 % of the determinations lie between 50 and 55 mg. %.
77 % of the determinations lie between 40 and 55 mg. %.

" Unlaked " Filtrates.

32 % of the determinations lie between 35 and 40 mg. %.
80 % of the determinations lie between 30 and 45 mg. %.

The differences between " laked " and " unlaked " vary between 3 % and 34 %, with an average of 18 %.

TOTAL NITROGEN.

Minimum-maximum variation, 2·4–3·2 gm. N %.
Average, 2·8 gm. N %.

TABLE 71.—*Non-Protein Nitrogen mg. % (N.P.N.) (" Laked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	15·9	26 6	—	—	24 9	17·9	23 7	22 7	19·6	—	17·7	—	—	27 7	21·1
31662	34·5	26 3	—	32·0	34·7	25·1	25 9	—	—	—	—	—	—	—	—
31742	20·5	29·2	—	—	24 9	—	25·6	26·8	18 3	—	20 7	—	—	37·5	23·9
31905	29·6	27·9	—	33·2	25·3	24·8	18·5	—	—	—	—	—	—	—	—
32176	30·5	28 8	—	—	27 7	30·5	23·8	23·0	19·5	—	17·9	—	—	27·9	22·9
Av...	26·1	27·8	—	32·4	26·6	24·6	23·9	23·9	19 0	—	18·7	—	—	31·0	22·7

TABLE 72.—*Non-Protein Nitrogen N.P.N. mg. % (" Unlaked ")*.

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	11·5	20·3	—	—	15·7	11·8	16·7	13·4	12·5	—	11·5	—	—	21·6	13·7
31662	23·3	20·6	—	22·6	26·2	16·1	18·1	—	—	—	—	—	—	27·0	15·4
31742	12·5	22·7	—	—	14·1	—	14·7	15·4	9·8	—	16·7	—	—	—	—
31905	18·1	23·1	—	26·1	18·7	16·7	11·3	—	—	—	—	—	—	21·8	15·1
32176	18·7	21·3	—	—	17·0	22·9	14·6	13·3	10·9	—	12·6	—	—	—	—
Av...	17·0	21·6	—	23·8	17·4	16·9	13·8	13·9	11·1	—	13·5	—	—	23·5	14·6

" Laked " Filtrates.

Minimum-maximum variation, 14·28–37·5 mg. N %.
Average, 25 mg. N %.

" Unlaked " Filtrates.

Minimum-maximum variation, 9·43–27·0 mg. N %.
Average, 16 mg. N %.
Average difference, 9 mg. N %.

The following table indicates the distribution:—

" Laked " Filtrates.		*" Unlaked " Filtrates.*	
mg. %.	Occurrence.	mg. %.	Occurrence.
Below 20	11	Below 10	1
20–25	21	10–15	22
25–30	25	15–20	20
30–35	7	20–25	15
above 35	1	25–30	4

" Laked " Filtrates.

38 % of the determinations lie between 25 and 30 mg. N %.
70 % of the determinations lie between 20 and 30 mg. N %.

" Unlaked " Filtrates.

35 % of the determinations lie between 10 and 15 mg. N %.
68 % of the determinations lie between 10 and 20 mg. N %.

The differences between the " laked " and " unlaked " filtrates vary from 19 % to 47 %, with an average of 36 %.

TABLE 73.—*Urea Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	4·4	12·7	—	—	8·6	5·2	7·6	6·3	2·9	—	4·9	—	—	13·8	5·6
31662	10·3	11·3	—	10·8	10·9	10·8	6·5	—	—	—	—	—	—	19·6	—
31742	6·3	12·3	—	—	6·2	—	5·6	6·8	2·9	—	6·1	—	—	19·6	5·3
31905	6·4	10·2	—	8·3	8·9	8·9	5·2	—	—	—	—	—	—	—	—
32176	7·4	10·7	—	—	9·2	15·5	7·9	4·3	1·7	—	4·6	—	—	13·7	6·5
Av...	6·9	10·5	—	10·0	8·4	10·1	6·5	5·4	2·7	—	5·2	—	—	15·7	5·8

" Laked " Filtrates.

Minimum-maximum variation, below 1·80–19·62 mg. N %.
Average, 9·6 mg. N %.

The following table indicates the distribution of the " laked " figures:—

	Occurrence.
Below 4	7
4– 5	7
5– 6	6
6– 7	8
7– 8	5
8– 9	8
9–10	4
10–11	5
above 11	9

36 % of the determinations lie between 4 and 7 mg. N %.

TABLE 74.—" *Total* " *Creatinine Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	2·0	2·4	—	—	2·1	2·0	1·8	2 4	2·2	—	2·1	—	—	1·8	2·0
31662	2·4	2·8	—	2·2	2·1	2·2	2·2	—	—	—	—	—	—	2·4	2·3
31742	2·1	2·6	—	—	2·0	—	2 0	2 4	2·1	—	2·0	—	—	2·4	2·3
31905	2·3	2·7	—	2·1	2·0	2·0	1·9	—	—	—	—	—	—	2·4	2·5
32176	2 2	2·7	—	—	2·1	1·9	1·9	2·1	2·0	—	2·0	—	—	2·4	2·5
Av...	2·2	2 6	—	2·2	2·1	2·0	2 0	2·3	2·1	—	2·0	—	—	2·2	2·3

TABLE 75.—" *Total* " *Creatinine Nitrogen mg. % (" Unlaked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	1·8	1·8	—	...	1·5	1·4	1 4	1·8	1·7	—	1·7	—	—	1·5	1·8
31662	1·9	2·1	—	1 8	1·6	1 4	1 1	—	·	—	—	—	—	2·0	1·6
31742	1·9	2 2	...	...	1 6	—	1·6	1 8	1·6	—	1·6	—	—	2·0	1·6
31905	1·9	2 0	—	1·5	1·6	1 6	1·4	—	—	—	—	—	—	2·0	1·9
32176	1·9	2·0	—	—	1 8	1·6	1·5	1·8	1 6	—	1·8	—	—	2·0	1·9
Av...	1·9	2·0	—	1 7	1·6	1·5	1·4	1 8	1·6	—	1·7	—	—	1 9	1·8

" *Laked* " *Filtrates.*

Minimum-maximum variation, 1·67–2·81 mg. N %.
Average, 2·18 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, 0·93–2·32 mg. N %.
Average, 1·73 mg. N %.
Average difference, 0·45 mg. N %.

The differences of the " laked " and " unlaked " " T ".C.N. figures vary between 4 % and 44 %, with an average of 21 %.

TABLE 76.—*Uric Acid Nitrogen mg. % (" Laked ").*

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	·26	·29	—	—	·22	·28	·24	—	26	—	·19	—	—	·39	·30
31662	·39	·38	—	·31	·23	·32	·30	—	—	—	—	—	—	—	—
31742	·30	·36	—	—	·24	—	·24	·27	·31	—	22	—	—	·44	·37
31905	·27	·31	—	·29	·35	·32	·23	—	—	—	—	—	—	—	—
32176	·31	·28	—	—	·23	·30	·24	·29	34	—	·30	—	—	·34	·25
Av...	·30	·33	—	·33	·25	·31	·26	·28	·30	—	·24	—	—	·39	·31

" *Laked* " *Filtrates.*

Minimum-maximum variation, Less than ·10–·44 mg. N %.
Average, ·29 mg. N %.

" *Unlaked* " *Filtrates.*

Minimum-maximum variation, Less than ·10–·20 mg. N %.

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TABLE 77.—*Amino-Acid Nitrogen mg. %* ("*Laked*").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	7·2	6 0	—	—	6·2	7·1	6·7	5·4	5·6	—	6·5	—	—	6·8	5·4
31662	6·5	6·7	—	8·4	8·2	6·0	8·2	—	—	—	—	—	—	—	—
31742	6 0	6 5	—	—	7·3	—	6·3	4·7	5·1	—	7·5	—	—	8·5	6·8
31905	6·0	6·7	—	7·4	7·4	6·0	6·4	—	—	—	—	—	—	—	—
32176	6·7	6·0	—	—	7·4	6·6	6·1	5·3	5·8	—	5·8	—	—	6·2	5·7
Av...	6·5	6 3	—	8·0	7 2	6·4	6·9	5·1	5 5	—	6·6	—	—	7·2	5·9

TABLE 78.—*Amino-Acid Nitrogen mg. %* ("*Unlaked*").

Sheep No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
28437	5·4	5·2	—	—	4·9	4·9	4·9	3·9	3·9	—	4·4	—	—	5·8	4·8
31662	5·3	5·0	—	5·4	5·6	4·0	5·1	—	—	—	—	—	—	—	—
31742	4·0	5·0	—	—	5·3	—	4·4	2·8	3·6	—	4·5	—	—	5·4	5·3
31905	5·2	5·2	—	4·5	5·3	4 4	5·0	—	—	—	—	—	—	—	—
32176	5·3	5 4	—	—	5·1	4·5	4·1	3·7	4·8	—	5·0	—	—	5·4	4·9
Av...	5·1	5·1	—	5·2	5·2	4·4	4·8	3·5	4 0	—	4 6	—	—	5·5	5·0

"*Laked*" *Filtrates.*

Minimum-maximum variation, 4·19–7·14 mg. N %.
Average, 6·39 mg. N %.

"*Unlaked*" *Filtrates.*

Minimum-maximum variation, 2·80–5·79 mg. N %.
Average, 4·85 mg. N %.
Average difference, 1·54 mg. N %.

The difference of the "laked" and "unlaked" figures vary from 14 % to 47 % with an average of 30 %.

(D) GENERAL COMPARISON OF GROUPS A, B, C, D AND E.

Hæmoglobin (Hb.).—It seems that no seasonal variations take place.

The Hb. content of the young lambs (B) and young ewes (D) are the lowest, while that of the 6-tooth sheep are the highest (C and E) (A. 14·7, B. 13·7, C. 16·7, D. 13·2, E. 17·6 gm. per 100 c.c.).

Sugar.—Green fodder was excluded from the ration in winter, being not available, and the blood sugar level of the sheep increased in the case of the sheep of the four older groups (A, C, D, E). That the sugar content was affected by the addition of green fodder towards December (1932), and the withdrawal again after the analyses were done, is evident from Graph III, but a definite conclusion cannot be drawn, since in Group A the sugar decreased in the "laked" filtrate from September to December (1932) and increased in the "unlaked" filtrate. From December (1932) the sugar decreased in both filtrates. On the contrary in Groups D and E it decreased in both filtrates from September to December and increased towards January (1933). In Group C the "laked" level increased from September to December (1932) and decreased to January (1933), while the sugar in the "unlaked" filtrate decreased over the whole period. (For A and B see Graph III, for C, D and E Tables 36, 37, 53, 54, 69 and 70.)

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The blood sugar level of the young lambs (B) is high but decreased during the first 6-7 months. The sugar content fell gradually showing occasional rises during the period of transition from a higher to a lower level.

The following figures are given for comparative purposes:—

	mg. %.		mg. %.
A av. L	43·3	U	35·2
B av. L	57·6	U	50·9
C av. L	46·2	U	36·8
D av. L	48·4	U	38·0
E av. L	48·4	U	39·6

The sugar levels of Groups A, C, D and E are fairly close to each other, A being the lowest by a few mg. %. The young lambs (B) however, have a relatively much higher blood sugar level.

The " laked " and " unlaked " curves both show the same general tendencies (Graph III.)

The average percentage difference of the " laked and " unlaked " figures differ little in the different groups except in Group B which is the lowest.

(A, 19 %; B, 12 %; C, 20 %; D, 20 %; E 18 %.)

Individual differences amongst sheep of the same sex and age have been noted in all the various groups (c.f. Tables 8, 9, 22, 23, 36, 37, 53, 54, 69 and 70.)

Total Nitrogen.—No seasonal variations occur with this constituent.

The figures for younger sheep are lower than those of the old ewes. (A, 3·0; B, 2·8; C, 2·9; D, 2·75; E, 2·8 gm. per 100 c.c.). Here also individual differences amongst sheep of the same sex and age have been noted in all the various groups.

Non-Protein Nitrogen (N.P.N.).—The N.P.N. content of blood is very much affected by the nature of the diet particularly when green fodder is supplied or not (vide Tables 10, 11, 24, 25, 38, 39, 55, 56, 71, 72, and Graph IV).

From September to December when green forage was supplied it rose in all the groups but decreased again during January after such forage had been withdrawn for about a month. The N.P.N. decreased during the winter months (March to July, see Graph IV).

The N.P.N. do not differ to a notable extent in the five groups:

	mg. N %.		mg. N %.
A av. L	24	U	16
B av. L	23	U	15
C av. L	26	U	19
D av. L	26	U	18
E av. L	25	U	16

The " laked " and " unlaked " N.P.N. curves have usually the same tendencies and run generally parallel to each other (Graph IV).

For comparative purposes the following average percentage differences of the average "laked" and "unlaked" figures for the five groups are stated:—

<div style="text-align:center">

A ... ... 34 %
B ... ... 35 %
C ... ... 27 %
D ... ... 31 %
E ... ... 36 %

</div>

Individual differences are also recorded in all the groups.

Urea Nitrogen.—The urea nitrogen curves generally run parallel with the N.P.N. curves (see e.g. Graph IV).

As the N.P.N. increases the urea nitrogen percentage of the N.P.N. increases and vice versa. This is just the opposite to what happens in the case of the amino-acids (see Graphs I and II).

The following averages afford a ready comparison between the various groups.

<div style="text-align:center">

A av. L 7·6 mg. N %
B av. L 6·6 mg. N %
C av. L 9·8 mg. N %
D av. L 9·3 mg. N %
E av. L 9·6 mg. N %

</div>

The average of Group B is the lowest, but it must be noted that the lambs were not included from the beginning of the experiment.
The content of urea nitrogen in the "laked" filtrate exceeds usually that in the "unlaked" filtrate, by a very small margin, mostly less than 1 mg. N %.

Individual differences have also been recorded for all the groups (see Tables 12, 26, 40, 57 and 73).

"Total" Creatinine Nitrogen.—As in the case of the other constituents no definite seasonal variation can be noted (see Graphs I, II and IV, and Tables 13, 14, 27, 28, 41, 42, 58, 59, 74 and 75.)

Again the variations, in the latter part of the experiment where the green fodder was added and later withdrawn from the ration, should be noted to emphasize the influence of diet on composition.

The percentage nitrogen of the N.P.N. tends to change reciprocally as the N.P.N. (in mg. %) varies.

The following averages are given for comparison:—

<div style="text-align:center">

	mg. N %.			*mg. N %.*
A L	... ... 2·14		U	... ... 1·63
B L	... ... 2·04		U	... ... 1·59
C L	... ... 2·17		U	... ... 1·79
D L	... ... 2·22		U	... ... 1·75
E L	... ... 2·18		U	... ... 1·73

</div>

The following are the percentage differences between the averages of the "laked" and "unlaked" figures: A, 24·5 %; B, 23·0 %; C, 18·0 %; D, 21 %; E, 21 %.

<div style="text-align:center">

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Uric Acid Nitrogen.—No definite seasonal variations can be noticed (Graph IV and Tables 15, 29, 43, 60 and 76).

The percentage nitrogen of the N.P.N. is fairly constant :—

(A 1·3, 1·3, 1·1, 1·0, 1·3, 1·1, 1·3, 1·3, ·57, 1·3.)
(B ·8, ·9, 1·2, 1·0, 1·2, 1·3, 1·0, 1·0.)
(C 1·3, ·9, 1·0, 1·2, 1·0, 1·4, ·51, 1·6.)
(D ·8, ·9, 1·2, 1·2, ·7, 1·6, 1·2, ·9, 1·1.)
(E 1·1, 1·2, ·9, 1·3, 1·1, 1·2, 1·6, 1·3, 1·3, 1·4.)

The following are the " laked " averages : A ·28, B ·20, C ·29, D ·36, E ·29. The average of Group B is definitely below that of the other groups.

Amino-Acid Nitrogen.—The blood amino acid levels do not change much towards winter, the levels in all groups however increasing towards December (1932) and with the exception of C decreased towards January (1933) (see Graph IV).

The percentage amino-acid nitrogen of the N.P.N. increases that of the urea nitrogen decreases, and the plotted curves consequently cross each other for a period during the winter months, most likely associated with the change in the ration. Thus the percentage amino-acid increases as the N.P.N. content of the blood decreases.

The following averages are given for comparison :—

	mg. N %.		mg. N %.
A L	6·15	U	4·43
B L	6·67	U	4·60
C L	6·37	U	4·57
D L	6·39	U	4·80
E L	6·55	U	4·85

In the case of " laked " filtrate of lambs the amino-acid content of blood is the highest, whereas for " unlaked " filtrate the highest average is that of Group E (wethers).

The following figures represent the percentage differences of the averages for the " laked " and " unlaked " filtrates : A 31, B 30·5, C 28·0, D 28·5, E 30·0.

DISCUSSION.

Sugar.—A possible explanation for the rise of sugar in blood during the winter may be that the animals at that time require more carbohydrate to cater for the necessary energy requirement and thus have a higher sugar content in the blood. This change may also be partly or wholly ascribed to the ration factor. Attention has been previously drawn to this aspect.

Folin (1930) states that his " unlaked " blood filtrates are free from non-sugar reducing substances. If this is the case the higher figures of the " laked " blood filtrates may be ascribed to one or more of the following three factors (Benedict, 1928) :—

(1) The presence of non-carbohydrates reducing substances which may affect the oxidation agent.

(2) The presence of non-glucose carbohydrate reducing substance (Hawk, 1931, p. 154).

(3) The presence of one or more substances in the blood filtrate which may cause a change in the copper complex so that the copper will be more easily reduced by the glucose or other reducing substances present (Hawk, 1931, p. 413; Benedict, 1929; Somogyi, 1927; 1930, 1931, etc.).

The first factor is usually regarded as the most essential, although Folin and Svedberg and Sjollema state that blood contains large quantities of non-glucose reducing carbohydrates. Thus it seems that special advantages are attached to the figures of the "unlaked" blood and that the figures are of more value than the "laked" blood sugar figures.

Only one explanation on the higher sugar content of lamb blood can at present be given, viz. the active metabolism going on in the young rapid-growing animals. Further research work on lamb blood is necessary to elucidate this problem.

Non-protein Nitrogen.—The non-protein nitrogen in the "laked" blood filtrate is always much higher than in the "unlaked" filtrate. The rest nitrogen in case of "unlaked" blood filtrate is always much less than in the "laked" filtrate, i.e. the sum of the urea nitrogen, "total" creatinine nitrogen, uric acid nitrogen and amino acid nitrogen is approximately the same as the non-protein nitrogen. Thus the undetermined nitrogen is small.

Lower amounts of non-sugar reducing substances go parallel with the lower nitrogen content in the various kinds of blood filtrates (Somogyi, 1930).

The amount of non-protein nitrogen varies according to the substances of which it is constituted, alterations in the urea level owing to the relatively large amount in the blood, particularly affecting the N.P.N. (Hawk, 1931, p. 415).

Urea Nitrogen.—The urea nitrogen is approximately the same in the two kinds of filtrates. As a rule when the urea nitrogen varies the non-protein nitrogen is correspondingly influenced.

An important phenomenon which has been noticed is that the urea nitrogen percentage of the N.P.N. falls, while the amino-acid percentage rises. While the essential catabolic end product of the proteins in the body is urea, one would not expect such a fall in urea nitrogen, particularly since the amino acid (in mg. N %) fraction is so remarkably constant.

The percentage curves of the other nitrogen-containing constituents are also inclined to change in the opposite direction to the urea nitrogen percentage curves, but the percentage nitrogen lost in the urea decrease is not made good through the other nitrogen-containing substances increasing, e.g. a larger percentage of the N.P.N. can be accounted for in November and December (1931) than in June, 1932 (Group A, Graph I). The percentage rest nitrogen calculated on the N.P.N. thus rises towards winter, although the rest nitrogen in mg. N % does not rise, but remains relatively constant. More accurate control and simultaneous analyses of blood, food and faeces would give one a better opportunity to be more definite about the deviations, but such research was not included in the original object of this work, viz. to furnish "normal" figures.

" Total " Creatinine Nitrogen.—The " total " creatinine nitrogen remains fairly constant over the whole period. The " unlaked " blood figures are relatively lower than the " laked " blood figures. Very little creatinine was found in sheep blood and it was found impossible to determine this constituent accurately.

Uric Acid Nitrogen.—The uric acid in the " unlaked " blood filtrate was usually above 50 % less than in the " laked " blood filtrate. Often the " unlaked " was undeterminably small. The uric acid nitrogen in " unlaked " blood filtrate is also extremely low.

Amino-Acid Nitrogen.—The amino-acid nitrogen remains fairly constant in both filtrates, but the figures in the " unlaked " blood filtrate are considerably lower than in the " laked " blood filtrate.

Finally, it must be noted that Group A includes three pregnant ewes, but since their figures do not show any definite difference as compared with the other three, it is not discussed here.

(E) COMPARISON WITH RESULTS OF OTHER WORKERS.

In spite of an extensive search in the available literature no data on the comparative values of " laked " and " unlaked " blood filtrates of sheep could be found. Even the normal data by any method are relatively scarce and fragmentary referring in many cases to analyses performed on one single sample of blood of a few animals and rarely more analyses on one and the same animal. No data extending over any time period of over 14 days or concerning age and sex was available. Furthermore, no nitrogen partition comprising *all* the constituents detailed in this paper has been found, the amino-acid nitrogen fraction particularly being generally omitted. If the history of the development of biochemical methods and the rapid strides made during the last decade in the evolution of a more accurate technique are considered, these deficiencies, or rather lack of more accurate and complete range of figures for the various constituents can be readily understood.

Below some figures gleaned from the available literature have been tabulated, together with my own data for comparison. In many cases these results were obtained by using different methods, with the result that an accurate comparison can hardly be made (Hawk, 1931; Host and Hatlehol, 1920, etc.). To give on example, the normal blood sugar figures for the human being obtained by using the following methods differ as follows :—

Folin-Wu method	90–120	mg. %
Folin-Malmros and Hagedorn-Jensen methods	75–105	,,
Folin's modification of Folin-Wu method ...	75–105	,,
Benedict Copper Reduction method	70–100	,,
Somogyi's method	70–100	,,

Sugar.

Year.	Author.	Method.	No. of Analyses.	Mg. %.	Remarks.
1911	Lyttkens and Sandgren (1911)	Bang	1	64	Plasma.
1923	Scheunert Pelchrzim (1923)	Folin & Wu.....	2 sheep	50·9–71·4 Av. 59·8	Blood "laked" filtrate
1927	Kleineberger Abderhalden, Bodansky (1927)	—	—	54–61 70 70	
1929	Volker (1929).....	Folin-Wu, Hagedorn–Jensen	4	44–61 Av. 51·8	Blood.
1929	Norris & Chamberlin ('29)	Maclean........	Slaughter house blood 64	42–118 Av. 61	64 sheep bled once. Wethers, ewes, lambs.
1933	Graf (1933).......	Folin & Svedberg	20 anal.	37–55·5 Av. 47·4	"Laked" blood.
1933	Graf.............	„	20 anal.	31·3–50·5 Av. 39.	"Unlaked" blood.
1933	Hamersma........	„	46 anal.	42·7–79·4 Av. 57·6	3 lambs, "laked" blood.
1933	„	„	46 anal.	35·8–70·9 Av. 50·8	"Unlaked" blood.
1933	„	„	6 sheep 80 anal.	27·0–59·5 Av. 43·3	Ewes "laked" blood.
1933	„	„	6 sheep 83 anal.	19·6–51·3 Av. 35·1	Ewes "unlaked" blood.
1933	„	„	3 sheep 33 anal.	34–74 Av. 46·2	"Laked" blood, 6 tooth ewes.
1933	„	„	3 sheep 33 anal.	23–61 Av. 36·8	6 tooth ewes "unlaked" blood.
1933	„	„	6 lambs 84 anal.	34–81 Av. 48·4	Ewe lambs "laked" blood.
1933	„	„	6 lambs 84 anal.	29–63 Av. 38·05	Ewe lambs "unlaked" blood.
1933	„	„	5 sheep 65 anal.	36–84 Av. 48·4	6 tooth wethers "laked" blood.
1933	„	„	5 sheep 66 anal.	27–53 Av. 39·6	6 tooth wethers "unlaked" blood.

As will be noticed from the above table the blood sugar content of sheep under South African conditions is lower than that found else where. Unfortunately diet and ages have not been specified in most cases and therefore a detailed comparison is not possible. But it will be noted that Scheunert and Pelchrzim's hay-fed sheep approximate our blood sugar figures. Other figures given by them (not incorporated here) indicate clearly the influence of diet on the blood sugar content, e.g. sheep receiving a diet of hay, yeast, mealies and cocoanut cake (amounts not stated) shows figures up to 89·9 mg. %. It is probable, therefore, that the generally higher level found elsewhere is associated with the better quality of food fed; furthermore, the methods, as already stated, may also play a rôle.

Non-Protein Nitrogen.

Year.	Author.	Method.	Number of Analyses.	Mg. N Percentage.	Remarks.
1913	Folin & Denis....	Folin & Denis..	Sheep	28	
1923	Scheunert & Pel-chrzim	Folin & Wu....	2 sheep 7 anal.	24–42·9 Av. 31·2	Fed with hay.
1929	Norris & Chamber-lin	Folin	25 sheep bled once	19·25–40·7 Av. 28·2	Ewes and wethers..
1929	,,	,, ...	26 lambs bled once	14·48–38·2 Av. 28·8	Lambs.
1933	Graf..............	Folin–Wu.......	9 sheep 20 anal.	13·6–20·0 Av. 16·5	Adult Sheep. L Jul. and Aug.
1933	,, ...	,, ...	9 sheep 20 anal.	9·4–15·3 Av. 12·5	U Jul. and Aug.
1933	Hamersma........	,, ...	6 sheep 86 anal.	13·39–48·4 Av. 24·0	Adult Sheep L.
	,, ...	,, ...	6 sheep 83 anal.	8·72–39·4 Av. 16·2	Adult ewes U.
	,, ...	,, ...	3 lambs 46 anal.	14·85–32·4 Av. 23	L. blood.
	,, ...	,, ...	3 lambs 46 anal.	9·31–25·8 Av. 14·5	U. blood.
	,, ...	,, ...	3 sheep 28 anal.	20·98–39·0 Av. 26	6 tooth ewe " laked " blood.
	,, ...	,, ...	3 sheep 29 anal.	10·7–27·4 Av. 19	6 tooth ewe " unlaked " blood.
	,, ...	,, ...	6 lambs 81 anal.	14·6–43·2 Av. 26	6 ewe lambs " laked " blood.
	,, ...	,, ...	6 lambs 80 anal.	8·5–35·3 Av. 18	Ewe lambs " unlaked " blood.
	,, ...	,, ...	5 sheep 65 anal.	14·28–37·5 Av. 25	6 tooth wethers " laked" blood.
	,, ...	,, ...	5 sheep 62 anal.	9·4–27·0 Av. 16	6 tooth wethers " un-laked " blood.

The striking fact here is that under South African conditions the non-protein nitrogen is on the whole so exceptionally low. This is again probably associated with the ration and climatic environment (drought). The figures obtained by Norris and Chamberlin were gathered under what must be considered unfavourable conditions, the blood being collected at a slaughter-house and, therefore, representing " mixed " blood, i.e. both venous and arterial. Furthermore, the time interval before analyses (3 hours) was too long to obtain accurate results. This can be clearly seen when adding the nitrogen containing constituents which, in some cases, then approximate the non-protein nitrogen figures although " total " creatinine and amino-acid nitrogen are not even included, i.e. the N.P.N. in these cases must be too low or one or other constituent, probably urea, must be too high, or both alternatives may also be possible.

Urea Nitrogen.

Year.	Author.	Method.	Number of Analyses.	Mg. N Percentage.	Remarks.
1913	Folin & Denis....	Folin & Denis..	Sheep	13·0	
1923	Scheunert & Pel-chrzim	Folin–Wu.......	2 sheep 7 anal.	7·5–23·1 Av. 12·0	
1929	Norris & Chamber-lin	Maclean.......	55 Sheep once each	6·26–21·8 Av. 12·8	Wethers, ewes and lambs.
1933	Graf.............	Folin & Svedberg	9 sheep 20 anal.	3·1–7·3 Av. 4·9	"Laked" blood. Jul. and Aug.
1933	„	„	9 sheep 20 anal.	3·0–7·3 Av. 4·7	"Unlaked" blood. Jul. and Aug.
1933	Hamersma........	„	6 sheep 80 anal.	1·85–31·5 Av. 7·56	"Laked" blood. Adult ewes.
	„	„	6 sheep 80 anal.	Less than 1·5–31·5	"Unlaked" blood. Adult ewes.
	„	„	3 lambs 45 anal.	Less than 1·5–17·69 Av. 6·6	"Laked" blood.
	„	„	3 lambs 45 anal.	Less than 1·5–18·05	"Unlaked" blood.
	„	„	3 sheep 28 anal.	1·5–20·78 Av. 9·83	6 tooth ewe. "Laked" blood.
	„	„	3 sheep 28 anal.	1·5–17·24	"Unlaked" blood. 6 tooth ewes.
	„	„	6 lambs 84 anal.	1·5–21·48 Av. 9·28	Ewe lambs. "Laked" blood.
			6 sheep 84 anal.	1·5–21·87	Ewe lambs. "Unlaked" blood.
	„	„	5 sheep 59 anal.	1·5–19·62 Av. 9·6	"Laked" blood. 6 tooth wethers.
	„	„	5 sheep 59 anal.	1·5–19·84	6 tooth wethers. "Unlaked" blood.

As the South African N.P.N. figures are relatively low, the logic conclusion is that the urea nitrogen must also be low, since this N fraction is normally responsible for from 13 % to over 50 % of the N.P.N. nitrogen.

Uric Acid Nitrogen.

Year.	Author.	Method.	Number Analyses.	Mg. N Percentage.	Remarks.
1913	Folin & Denis ([1])..	Folin & Denis..	Sheep	·05	
1914	Steinitz..........	—	Sheep	1·0–1·5	
1929	Norris & Chamberlin	Benedict........	34 sheep each once	·71–1·72	Wethers.
1929	„	„	28 lambs each once	·90–1·56	
1933	Graf.............	Folin..........	9 sheep 20 anal.	·15–·26 Av. ·22	"Laked" blood.
1933	„	„	9 sheep 20 anal.	·10–·22 Av. ·13	"Unlaked" blood.
1933	Hamersma........	„	6 sheep 68 anal.	Less than ·10–·48 Av. ·28	"Laked" blood. Adult ewes.
1933	„	„	6 sheep 68 anal.	Less than ·10–·30	"Unlaked" blood. Adult ewes.
1933	„	„	3 lambs 45 anal.	Less than ·10–·43 Av. ·20	"Laked" blood.
1933	„	„	3 lambs 45 anal.	Less than ·10–·24	"Unlaked" blood.
1933	„	„	3 sheep 28 anal. 45 anal.	Less than ·08–·52 Av. ·29	6 tooth ewes. "Laked" blood.
1933	„	„	3 sheep 28 anal.	Max. ·19	6 tooth ewes. "Unlaked" blood.
1933	„	„	6 lambs 84 anal.	Less than ·08–·52 Av. ·36	Ewe lambs. "Laked" blood.
1933	„	„	6 lambs 84 anal.	Max. ·24	Ewe lambs. "Unlaked" blood.
1933	„	„	5 sheep 59 anal.	Less than ·08–·44 Av. ·29	6 tooth wethers. "Laked" blood.
1933	„	„	5 sheep 59 anal.	Max. ·20	6 tooth wethers. "Unlaked" blood.

With the exception of (I) less uric acid has been found under South African conditions than has been found by other workers. During the analyses it was found that partly coagulated blood was higher in uric acid nitrogen (e.g. 1·3 mg. N %), but such blood was always discarded.

(F) GENERAL SUMMARY.

1. "Laked" and "unlaked" blood filtrates of 23 sheep of various ages were analysed.

2. The blood analyses were done over a period of 15 months in the case of 20 sheep, and 11 months in the case of 3 lambs. The same animals have always been used during the stated period.

3. Determinations of all the groups have been made for haemoglobin, total nitrogen, urea nitrogen, "total" creatinine nitrogen, uric acid nitrogen and amino-acid nitrogen in the two blood filtrates respectively.

4. The normal range and the average of each constituent, together with the average difference %, etc., of all the groups are given.

5. Graphs of two groups (A and B) of the 5 groups illustrate the averages of all the constituents (except Hb, and T.N.) of the blood over the stated periods; and other graphs the nitrogen containing substances (except T.N.) expressed as per cent. of the N.P.N.

6. Comparisons of the results with those of other workers are included.

(G) ACKNOWLEDGMENTS.

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REFERENCES.

BEHRE, J. A., AND BENEDICT, S. R. (1922). Studies in creatine and creatinine metabolism IV. On the question of the occurrence of creatinine and creatine in blood. *Jnl. Biol. Chem.*, Vol. 52, p. 11.

BENEDICT, S. R. (1929). A note on the purification of picric acid for creatinine determination. *Jnl. Biol. Chem.*, Vol. 82, p. 1.

BENEDICT, S. R. (1928). The determination of blood sugar. *Jnl. Biol. Chem.*, Vol. 76, p. 457.

BENEDICT, S. R., AND NEWTON, E. B. (1929). Studies on the non-sugar reducing substances of the blood and urine. I. Glutathione and Thioneine in blood. *Jnl. Biol. Chem.*, Vol. 83, p. 361.

BENEDICT, S. R. (1931). The analyses of whole blood I. The precipitation of the proteins. *Jnl. Biol. Chem.*, Vol. 92, p. 135.

BENEDICT, S. R. (1931). The analyses of whole blood III. Determination and distribution of uric acid. *Jnl. Biol. Chem.*, Vol. 92, p. 161.

FEIGL, J. (1921). A study of N.P.N. of human blood II. Chemical Pathology of the urea fraction with a critical study of methods. *Zeitsch. exp. Med.*, Vol. 12, pp. 55-133.

FEIGL, J. (1920). The presence of creatinine and creatine in blood of normal and pathological conditions IV. *Biochem. Z.*, Vol. 105, pp. 255-281.

FOLIN, O. (1930). "Unlaked" blood as a basis for blood analysis. *Jnl. Biol. Chem.*, Vol. 86, p. 173.

FOLIN, O., AND SVEDBERG, A. (1930). Micro-methods for the determination of N.P.N. urea, uric acid, and sugar in laked blood. *Jnl. Biol. Chem.*, Vol. 88, p. 85.

FOLIN, O., AND WU, H. (1919). A system of blood analysis. *Jnl. Biol. Chem.*, Vol. 38, p. 81.

FOLIN, O. (1929). Two revised copper methods for blood sugar determination. *Jnl. Biol. Chem.*, Vol. 82, p. 83, and Vol. 88, p. 85.

FOLIN, O., AND SVEDBERG, A. (1930). An improved distillation method for the determination of urea in blood. *Jnl. Biol. Chem.*, Vol. 88, p. 77.

FOLIN, O., AND DENIS, W. (1916). Nitrogen determination by direct nesslerization. V. Urea in blood. *Jnl. Biol. Chem.*, Vol. 26, p. 505.

FOLIN, O. (1930). An improved method for the determination of uric acid in blood. *Jnl. Biol. Chem.*, Vol. 86, p. 179.

FOLIN, O. (1922). A system of blood analysis, supplement III. *Jnl. Biol. Chem.*, Vol. 57, p. 377.

FOLIN, O., AND SVEDBERG, A. (1926). The sugar in urine and in blood. *Jnl. Biol. Chem.*, Vol. 70, p. 405.

FOLIN, O., AND SVEDBERG, A. (1930). Diffusible non-protein constituents in blood and their distribution between plasma and corpuscles. *Jnl. Biol. Chem.*, Vol. 88, pp. 715-718.

FOLIN, O., AND DENIS, W. (1913). Protein metabolism from the standpoint of blood and tissue analyses. (6th paper.) On uric acid, urea and total non-protein nitrogen in human blood. *Jnl. Biol. Chem.*, Vol. 14, p. 29.

GAEBLER, O. H., AND KELTCH, A. K. (1928). On the nature of blood creatinine. *Jnl. Biol. Chem.*, Vol. 76, p. 337.

GAEBLER, O. H. (1930). Further studies of blood creatinine. *Jnl. Biol. Chem.*, Vol. 89, p. 451.

GREEN, C. H., SANDIFORD, K., AND ROSS, H (1923-4). The amino-acid content of the blood in normal and pathologic conditions. *Jnl. Biol. Chem.*, Vol. 58, p. 845.

HAWK, P. B., AND BERGEIM, O. (1931). *Practical physiological chemistry.* 10th Edition.

HOST, H. F., AND HATLEHOL, R. (1920). Blood sugar concentration and blood sugar methods. *Jnl. Biol. Chem.*, Vol. 42, p. 347.

HUBBARD, R. S., AND DEEGAN, J. K. (1930). Note on the action of the colon bacillus upon the non-glucose reducing substances in human blood. *Jnl. Biol. Chem.*, Vol. 86, p. 575.

LYTTKENS, H., AND SANDGREN, J. (1911). Ueber die Verteilung der reduzierenden Substanzen in Säugetierblut. *Biochem. Zeitsch.*, Vol. 36, p. 261.

MACLEOD, J. J. R. (1927). Kohlehydratstoffwechsel und Insulin.

NORRIS, J. H., AND CHAMBERLIN, W. E. (1929). A chemical and histological investigation in Victoria (Australia) of the blood of cattle and sheep. *Aust. Jnl. of Exp. Biol. and Med. Sc*, Vol. 6, p. 285.

NEWCOMER, H. S. (1919). *Jnl. Biol. Chem.*, Vol. 37, p. 465.

SCHEUNERT, A., AND VAN PELCHRZIM, H. (1923). Ueber den Gehalt des Blutes verschiedener Tierarten an Zucker u.s.w. *Biochem. Zeitsch.*, Vol. 139, p. 17.

SOMOGYI, M. (1927). Reducing non-sugar and true sugar in human blood. *Jnl. Biol. Chem.*, Vol. 75, p. 33.

SOMOGYI, M. (1930). A method for the preparation of blood filtrates for the determination of sugar. *Jnl. Biol. Chem.*, Vol. 86, p. 655.

SOMOGYI, M. (1931). The use of copper and iron salts for deproteinazation of blood. *Jnl. Biol. Chem.*, Vol. 90, p. 725: also note on the distribution of blood sugar, p. 731.

SOMOGYI, M. (1930). Nitrogenous substances in zinc filtrate of human blood. *Jnl. Biol. Chem.*, Vol. 87, p. 339.

STEINITZ (1914). *Zeitsch. f. Phys. Chem.*, Vol. 90, p. 108.

TERVAERT, D. G. COHEN (1920). Determination of micro-reduction. *Zeitsch. Phys. Chem.*, Vol. 110, pp. 41-54.

VÖLKER (1929). Blutzuckeruntersuchungen an gesunden und kranken Tieren. *Arch. Wiss Prak. Tierheilkunde*, Vol. 59, p. 16.

WU, H. (1922). Separate analyses of the corpuscles and plasma. *Jnl. Biol. Chem.*, Vol. 57, p. 21.

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Section VI.

Dips and Dipping.

Researches into Dips and Dipping.

A.   Lime-Sulphur Dips.

Paper IV.   Further Studies on the Colorimetric Method as a Rapid Means of Control of Polysulphide Solutions.*

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IN a previous study of the colorimetric method as applied to polysulphide solutions—Paper A. III.—it was shown that under certain conditions solutions of the polysulphides of calcium conform to Beers Law, that is that the colour intensity is a linear function of the concentration. It was also shown, however, that field conditions, especially the process of dipping, affect these solutions to such an extent that this relation between colour intensity and concentration no longer holds. As a result of rather intensive dipping all dips become markedly turbid, and of these a very large percentage retains their turbidity even after standing unmolested for some months. Apart from this, dipping invariably results in appreciable amounts of suint alkali constituents entering the dip with the result of a partial base exchange being effected. Thus not only the reaction of the solution is changed, but some of the calcium polysulphides are also replaced by alkali polysulphides.

As a result of these observations it was decided to study the effect of the reaction of the medium on the relation of colour intensity to polysulphide concentration. For this purpose the effect of both sodium hydroxide and sodium carbonate on the colour characteristics of the polysulphides of sodium were studied. A solution of sodium polysulphide was prepared by boiling a concentrated solution of sodium sulphide with excess flowers of sulphur, diluting this concentrate somewhat, filtering, and making up to a fairly large volume. In this way a stock solution containing 5.10 gm. polysulphide sulphur per 100 c.c. was obtained. This stock solution was protected against atmospheric oxidation by covering its surface with a layer of 70-80° benzine. For the experiment under consideration 5·0 c.c. of this stock solution were carefully pipetted into a 50 c.c. measuring flask containing a few cubic centimetres of benzine, the sides of the flask immediately rinsed down with distilled water, and then different

amounts of N/1 NaOH or 9 per cent. sodium carbonate (9 gm. Na_2CO_3—anhydrous—per 100 c.c.) added as required; the solutions were then made up to volume. The thus prepared solutions were allowed to stand at room temperature, and compared colorimetrically from time to time with the polysulphide solution to which no alkali had been added. After this the solutions were analysed by the volumetric cadmium acetate method (Paper II, A.).

Considering first the colorimetric analyses, the " apparent " polysulphide concentrations so obtained at different reaction times and different sodium hydroxide concentrations have been tabulated in Table I. All concentrations have been expressed in gm. poly-sulphide sulphur per 100 c.c. solution.

TABLE 1.
Effect of NaOH on $Na_2S_{4.6}$—Colorimetric.

C.c. N/1 NaOH added per 50 c.c.	After 19 hrs.		After 66 hrs.		After 115 hrs.		After 236 hrs.	
	Color. reading.	P.S.S. conc. (app.).	Color. reading.	P.S.S. conc. (app.).	Color. reading.	P.S.S. conc. (app.).	Color. reading.	P.S.S. conc. (app.).
0·0 c.c.....	20	0·510	20	0·510	20	0·510	20	0·510
0·1 c.c.....	21	0·485	22	0·463	22	0·463	22	0·463
0·4 c.c.....	25	0·408	28	0·364	29	0·352	28	0·364
1·0 c.c.....	30	0·340	35	0·290	36	0·284	38	0·268
2·0 c.c.....	32	0·318	37	0·275	40	0·255	43	0·238
10·0 c.c.....	36	0·284	45	0·226	48	0·212	52	0·196

P.S.S. = polysulphide sulphur.

These apparent polysulphide concentrations have been calculated from the colorimetric readings by assuming that the polysulphide concentration remains proportional to the colour intensity. Initially, however, all solutions contained the same concentrations, since in every case 5 c.c. of the same stock solution were diluted to 50 c.c. Hence, judging by the marked fall in the apparent polysulphide concentration—from 0·510 to 0·196—a distinct change in the nature of the initially present polysulphide must have occurred.

Since sodium hydroxide reacts with elementary sulphur according to the equation :—

$$6\ NaOH + (2x+2)\ S \rightarrow 2\ Na_2Sx + Na_2S_2O_3 + 3\ H_2O$$

forming sodium polysulphides and sodium thiosulphate, it may be expected that this alkali will also react with the sulphur of higher polysulphides forming lower polysulphides and thiosulphate, and consequently increasing the monosulphide sulphur equivalent. This type of reaction, which we shall in future refer to as Reaction I, may thus be represented by some of the following reactions :—

(1) $10\ Na_2S_5 + 6\ NaOH \rightarrow 12\ Na_2S_4 + Na_2S_2O_3 + 3\ H_2O.$

(2) $4\ Na_2S_5 + 6\ NaOH \rightarrow 6\ Na_2S_3 + Na_2S_2O_3 + 3\ H_2O.$

(3) $8\ Na_2S_5 + 6\ NaOH \rightarrow 2\ Na_2S_3 + 8\ Na_2S_4 + Na_2S_2O_3 + 3\ H_2O$
etc.
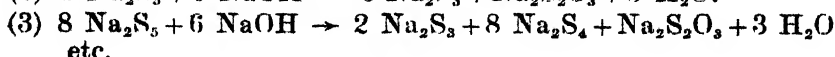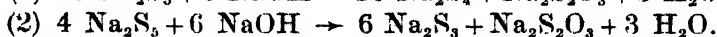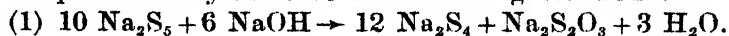
To what extent these reactions influence the figures tabulated in Table I may be determined by considering the analytical data tabulated in Table II.

TABLE II.

Effect of NaOH on $Na_2S_{1.6}$ analysed after 236 hours.

C.c. N/1 NaOH per 50 c.c.	Thiosul-sulphate (gm. S/100 c.c.).	M.S.S.E. (gm. S/100 c.c.).	F.P.S.S. (gm. S/100 c.c.).	Polysul-phide (gm. S/100 c.c.).	Total S (calculated) (gm. S/100 c.c.).	x (in Na_2Sx).
0·0 c.c.	0·020	0·111	0·399	0·510	0·530	4·60
0·1 c.c.	0·024	0·107	0·390	0·497	0·521	4·64
0·4 c.c.	0·031	0·113	0·387	0·500	0·531	4·42
1·0 c.c.	0·033	0·119	0·377	0·496	0·529	4·17
2·0 c.c.	0·045	0·120	0·368	0·488	0·533	4·07
10·0 c.c.	0·045	0·122	0·365	0·487	0·532	4·00

M.S.S.E. == Monosulphide sulphur equivalent.
F.P.S.S. == Free polysulphide sulphur (see previous reports).

From the figures recorded in the last column of the above table for the atomic ratio of sodium to sulphur we note that there is some decrease in the average order of the polysulphides. There is also a small corresponding increase in the thiosulphate and monosulphide sulphur equivalent. However, as has also been observed by Green (1915), the changes noted are small, the actual polysulphide concentration only decreasing from 0·510 to 0·487 gm. sulphur per 100 c.c. It is clear, therefore, that the chemical interaction described under Reaction I cannot explain why the apparent polysulphide concentration should, under the same conditions, fall from 0·510 to 0·196 gm. sulphur per 100 c.c.

To explain this observation, i.e. why the colour intensity of the polysulphides in solution decreases so markedly on adding sodium hydroxide, there would appear to be only two possible explanations. Either the sodium hydroxide, probably in dissociated form, is in some way or other linked on to the still unsaturated sulphur atoms of the polysulphide molecule forming a new complex compound with a much lower colour intensity, or the inorganic polysulphides in solution, like the organic polysulphides, are capable of existing in different isomeric forms with different colour intensities. The further discussion of this highly interesting observation, however, must be considered beyond the scope of the present paper.

Proceeding now to the effect of sodium carbonate on the poly-sulphides in solution, a similar state of affairs is revealed. Calculating again the apparent polysulphide concentrations from the colori-metric readings by assuming that the polysulphide concentration remains proportional to the colour intensity, we again find an appreciable fall in the apparent concentrations.

TABLE III.

Effect of Na_2CO_3 on $Na_2S_{4.6}$—colorimetric.

C.c. 9% Na_2CO_3 added per 50 c.c.	After 45 hours.		After 168 hours.		After 360 hours.	
	Color. reading.	P.S.S. conc. (apparent).	Color. reading.	P.S.S. conc. (apparent).	Color. reading.	P.S.S. conc. (apparent).
0·0 c.c.	20	0·510	20	0·510	20	0·510
0·1 c.c.	21	0·485	21	0·485	21	0·485
0·4 c.c.	21	0·485	22	0·463	23	0·443
1·0 c.c.	23	0·443	24	0·424	24·5	0·416
2·0 c.c.	24	0·424	26	0·392	29	0·352
10·0 c.c.	28	0·364	30	0·340	36	0·284

P.S.S. = Polysulphide sulphur.

Comparing this fall in apparent polysulphide concentration—from 0·510 to 0·284 gm. sulphur per 100 c.c.—with the actual change in the concentration as revealed by chemical analysis, we again find that the appreciable change in apparent concentration cannot be accounted for by the type of reaction grouped under Reaction I.

TABLE IV.

Effect of Na_2CO_3 on $Na_2S_{4.6}$—analysed after 360 hours

C.c. 9% Na_2CO_3 per 50 c.c.	Thiosulphate (gm. S/100 c.c.).	M.S.S.E. (gm. S/100 c.c.).	F.P.S.S. (gm. S/100 c.c.).	Polysulphide (gm. S/100 c.c.).	Total S (calculated) (gm. S/100 c.c.).	λ (in Na_2Sx).
0·0 c.c.	0·020	0·111	0·399	0·510	0·530	4·60
0·1 c.c.	0·030	0·111	0·390	0·501	0·531	4·51
0·4 c.c.	0·028	0·111	0·390	0·501	0·529	4·51
1·0 c.c.	0·030	0·113	0·386	0·499	0·529	4·41
2·0 c.c.	0·027	0·113	0·388	0·501	0·528	4·43
10·0 c.c.	0·042	0·114	0·380	0·494	0·536	4·33

M.S.S.E. = Monosulphide sulphur equivalent.
F.P.S.S. = Free polysulphide sulphur.

As a result of these observations on the effect of alkali on the colour of polysulphides, the practical application of the colorimetric principle was further studied by adding excess alkali to the calcium polysulphide solutions. For this purpose sodium carbonate was found most useful, since firstly it converts all polysulphide to alkali polysulphide, and secondly precipitates the matter in suspension along with the calcium carbonate. To make this clarification of the solution more complete some absolute alcohol was also added, the polysulphides of the alkali metals being soluble in alcohol.

For the purpose of studying the effect of this treatment on the colour intensity of the solutions, a calcium polysulphide stock solution of known composition was suitably diluted to give a series of polysulphide solutions ranging from 0·14 to 1·44 gm. polysulphide sulphur per 100 c.c. In making up these solutions the required amount of stock solution was pipetted into a 50 c.c. measuring flask containing 10 c.c. of a 9 per cent. sodium carbonate solution and 5 c.c. absolute alcohol, and the mixture immediately made up to 50 c.c. After shaking up thoroughly, the solution was immediately filtered through a dry filter into a dry 50 c.c. bottle containing a few c.c. of benzine. The thus prepared solutions were then immediately compared under the colorimeter against a set of standard pure potassium dichromate solutions. All concentrations have been expressed in gm. per 100 c.c. solution.

TABLE V.

Effect of Na_2CO_3 Treatment on Polysulphide Concentration.

Conc. polysulphide used as $CaS_{4\cdot6}$	Colorimeter setting.	Concentration of $K_2Cr_2O_7$ standard.	Colorimeter reading (average).	$K_2Cr_2O_7$ corresponding to polysulphide used.
0·14	20	0·03	40·2	0·06
0·14	20	0·06	17·0	0·05
0·29	20	0·06	31·0	0·09
0·29	20	0·09	22·0	0·10
0·44	20	0·09	37·0	0·17
0·44	20	0·12	26·0	0·16
0·57	20	0·12	39·0	0·23
0·57	20	0·18	28·0	0·25
0·72	20	0·18	36·5	0·33
0·72	20	0·24	30·0	0·36
1·00	20	0·24	41·0	0·49
1·00	20	0·30	36·0	0·54
1·15	20	0·30	38·5	0·58
1·15	20	0·42	33·5	0·70
1·44	20	0·42	42·5	0·89
1·44	20	0·54	33·0	0·89

Table V. thus gives the colorimetric relationship between polysulphide concentration and the corresponding potassium dichromate concentration over a range of polysulphide concentrations of 0·10 to 1·44 gm. sulphur per 100 c.c. solution.

Using this relationship, and treating a number of lime-sulphur dips obtained from the field in a similar manner, it was found that the polysulphide concentration of all the dips studied could be determined with a fair amount of accuracy. For this purpose 25 c.c. of the dip sample as received was pipetted into a 50 c.c. measuring flask containing 10 c.c. of 9 per cent. sodium carbonate and 5 c.c. absolute alcohol, the mixture made up to volume, shaken up, and immediately filtered through a dry filter into a dry bottle containing a few cubic centimeters of benzine. By thus decreasing the chances of oxidation to a minimum, a perfectly clear filtrate was obtained in every case. If, during the process of filtering, the filtrate is not

protected against the atmospheric oxygen, it often soon becomes turbid due to the formation of elementary sulphur. Laboratory experience has shown that polysulphide solutions, adulterated by the process of dipping, display a much greater sensitivity towards atmospheric oxygen than pure polysulphide solutions.

In the following table—Table VI—the colorimetric analyses obtained as above have been compared with the actual analyses obtained with the volumetric cadmium acetate method.

TABLE VI.

Colorimetric Analyses of Field Dips.

Dip.	$K_2Cr_2O_7$ standard used (gm./100 c.c.).	Polysulphide found colorimetrically (gm. S/100 c.c.).	Poylsulphide actually present (gm. S/100 c.c.).	x (in CaSx).
1..........	0·06	0·23	0·24	—
2..........	0·12	0·40	0·46	5·25
3..........	0·12	0·42	0·45	4·6
4..........	0·24	0·44	0·44	4·2
5..........	0·12	0·44	0·47	5·0
6..........	0·12	0·44	0·52	5·2
7..........	0·24	0·49	0·47	4·5
8..........	0·24	0·50	0·46	4·4
9..........	0·24	0·50	0·54	4·9
10..........	0·42	0·62	0·50	4·2
11..........	0·24	0·66	0·59	4·4
12..........	0·24	0·66	0·76	4·6
13..........	0·24	0·73	0·86	4·8
14..........	0·42	1·01	1·15	4·4
15..........	0·12	0·23	0·22	1·9
16..........	0·12	0·42	0·42	4·0
17..........	0·24	0·45	0·39	4·4
18..........	0·24	0·56	0·56	3·9
19..........	0·42	0·61	0·55	2·8
20..........	0·24	0·61	0·61	3·5
21..........	0·24	0·68	0·72	3·9
22..........	0·24	0·69	0·72	4·6
23..........	0·42	0·77	0·68	3·9
24..........	0·24	0·77	0·85	4·5
25..........	0·24	0·82	0·83	4·0

(Dips 1–14: Undipped Washes. Dips 15–25: Dipped Washes.)

The results show that for all purposes of field control the colorimetric method as here described gives quite satisfactory results. The accuracy of the method would seem to be influenced neither by the process of dipping nor by the atomic ratio of metal to sulphur in the dipwash analysed. If this finding can be substantiated on a still wider range of dip samples the main difficulty in the rapid analysis of lime-sulphur dip-washes has been overcome, although at this stage we are not in a position to give a full explanation of the observations made, since the true nature of lime-sulphur solutions, their behaviour on oxidation, etc., are all questions awaiting further intensive research. However, the possible adaptation of this colorimetric principle as a basis for evolving a simple field method of control is sufficiently promising to warrant its further study.

SUMMARY.

As a continuation of the preliminary study of the colorimetric method as a rapid means of control of polysulphide solutions, the effect of free alkali on sodium polysulphide solutions at room temperature was studied, since in the process of dipping the washing-out of the suint of the fleece results in the interaction of dipwash with alkali and in the formation of alkali polysulphides. It was found that the chemical interaction, as revealed by ordinary chemical analysis, is slight, though the colorimetric study reveals a marked change in chemical nature. This change was ascribed, either to the formation of a complex compound between the alkali and the polysulphide, or to the existence of different isomers of the polysulphides in solution. The observation that field samples of lime-sulphur cannot be directly compared colorimetrically is thus partially explained.

It was further shown that the addition of excess sodium carbonate and alcohol causes immediate precipitation of all turbidity-forming materials, rendering a perfectly clear solution of the alkali polysulphides which can be matched against a potassium dichromate standard. In this way fairly accurate values were obtained with both used and unused lime-sulphur dip washes. It is proposed to apply this colorimetric principle for evolving a simple field method of control.

REFERENCES.

GRAF, H., and JORDEN, T. J. WILKEN (1932). General Introduction. Lime-Sulphur Dips 18th Rept. Dir Vety. Serv. and Anim. Indust. Paper A.I, p. 1005.

GREEN, H. H. (1915). Upon composition and analyses of polysulphide solutions. 3rd and 4th Rept. Dir. Vety. Res., p. 192.

JORDEN, T. J. WILKEN (1932). A preliminary study of a colorimetric analysis. 18th Rept. Dir. Vety. Serv. and Anim. Indust. Paper A.II, p. 1015.

JORDEN, T. J. WILKEN (1932). A Preliminary study of a colorimetric method as a rapid means of control of polysulphide solutions. 18th Rept. Dir. Vety. Serv. and Anim Industr., p. 1029.

Researches into Dips and Dipping.

A. Lime-Sulphur Dips.

Paper V. The minimum effective concentration of Lime-Sulphur Dips for Sheep scab eradication.

By

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THE official strength of lime sulphur dips, viz., 1·5 per cent. sulphide sulphur—recommended by the Union Department of Agriculture seems to have been adopted as a result of the specifications of the American Bureau of Animal Industry. In the American B.A.I. Order No. 263 of 1st July, 1919, it is specified that lime-sulphur baths shall be maintained at a strength of not less than $1\frac{1}{2}$ per cent. of "sulphid sulphur". Regarding the lowest effective strength it is interesting to note thereanent the precise wording of the Order (pp. 19-20): "No dip other than the lime-sulphur or the nicotine dip will hereafter be given Department permission for use in official dipping for scabies, unless it has been shown to the satisfaction of the Bureau (1) that the strength of the bath prepared therefrom may be satisfactorily determined in the field by a practical portable testing outfit; (2) that under actual field conditions the dipping of cattle in a bath of definite strength will effectually eradicate scabies infection without injury to the animals dipped". From this one is led to conclude that the Bureau considered any appreciable deviation from this specified strength of 1·5 per cent. as a rather serious matter. Whether this attitude on the part of American authorities has subsequently been changed in any way, we have not been able to determine.

However, certain experiments carried out at Onderstepoort in 1915 by Bedford and Green with soda-sulphur (sodium polysulphide) solutions showed that the minimum effective concentration against sheep scab must be somewhere between 0·31 and 0·64 per cent. polysulphide sulphur. In a Bulletin issued by the Union Minister of Mines and Industries, Green (1919) suggested that the American standard of 1·5 per cent. sulphide sulphur for sheep scab was empirical and in all probability unnecessarily high. A strength of 1 per cent. was suggested as adequate.

In the following experiment, conducted at Onderstepoort, an attempt was made to determine the actual minimum effective concentration. The sheep were dipped individually, each animal being kept immersed for a full two minutes and its head ducked twice during this operation. A second dipping was given nine days after the first. In every case the ears were thoroughly hand-treated with a mixture consisting of two parts linseed oil to one part paraffin. The different groups were isolated in different pens, care being taken not to put a dipped group back into a still infected pen.

EXPERIMENT No. 4589.

Test No. 1.

10.2.1932. The following six sheep badly infected with scab dipped in Capex Lime-sulphur (strength 0·92 per cent. polysulphide sulphur): Nos. 31158, 31589, 31831, 31951, 31999 and 32100.

19.2.1932. Second dipping. Strength 0·85 per cent. polysulphide sulphur.

20.2.1932. Sheep No. 32100 died of general debility due to scab infection.

Result.—No scab parasites were found on the sheep either after the first dipping or up to six months after the second dipping.

Test No. 2.

10.2.1932. The following six sheep badly infected with scab dipped in Capex Lime-sulphur (strength 0·72 per cent. polysulphide sulphur): Nos. 31690, 31695, 32083, 32144, 32276 and 32354.

13.2.1932. Sheep No. 32354 died.

20.2.1932. Second dipping. Strength 0·77 per cent. polysulphide sulphur.

Result.—No scab parasites were found on the sheep either after the first dipping or up to six months after the second dipping.

Test No. 3.

16.2.1932. The following six sheep badly infected with scab dipped in Capex Lime-sulphur (strength 0·47 per cent. polysulphide sulphur): Nos. 31141, 31143, 31665, 31781, 32166 and 32314.

25.2.1932. Second dipping. Strength 0·47 per cent. polysulphide sulphur.

1.3.1932. Sheep No. 31143 died as a result of scab infection and myiasis.

Result.—No scab parasites were found on the sheep either after the first dipping or up to six months after the second dipping.

Test No. 4.

5.4.1932. The following six sheep badly infected with scab dipped in Capex Lime-sulphur (strength 0·29 per cent. polysulphide sulphur): Nos. 31488, 31645, 31715, 31922, 32028 and 32200.

14.4.1932. Second dipping. Strength 0·31 per cent. polysulphide sulphur.

Result.—No scab parasites were found on the sheep either after the first dipping or up to six months after the second dipping.

Test No. 5.

1.6.1932.　The following six sheep badly infected with scab dipped in Capex Lime-sulphur (strength 0·2 per cent. polysulphide sulphur): Nos. 27589, 28700, 31166, 31540, 31967 and 32133.

6.6.1932.　A few live acari found on each of the sheep.

10.6.1932.　Second dipping. Strength 0·2 per cent. polysulphide sulphur.

12.7.1932.　All sheep found to be infected.

　Result.—The dip did not cure the sheep of scab.

Test No. 6.

13.7.1932.　The following 16 sheep, badly infected with scab, dipped in Capex Lime-sulphur (strength 0·25 per cent. polysulphide sulphur): Nos. 31269, 31436, 31463, 31500, 31514, 31516, 31519, 31536, 31581, 31616, 31650, 31766, 31793, 31909, 32091 and 32323.

　Sheep No. 31269 died after dipping.

22.7.1932:　Second dipping. Strength 0·3 per cent. polysulphide sulphur.

Result.—The dip failed to cure the sheep of scab. Acari were found on the sheep at various periods after they had been dipped. On a few of the animals only fresh lesions were found, indicating that they had become reinfected through coming in contact with the infected sheep.

SUMMARY AND CONCLUSIONS.

The above tests demonstrate that lime-sulphur dips used at a strength varying between 0·9 and 0·3 per cent. polysulphide sulphur may be effective in curing sheep of scab when badly infected. When used at a lower concentration it was found to be ineffective. In one test, however, in which the strength of the first dip was 0·25 per cent. and the second dip 0·3 per cent. polysulphide sulphur, some of the sheep were apparently cured of the disease as only fresh lesions could be found on them, indicating that they had become reinfected through contact with others which were not cured. It is obvious, therefore, that the dip, when used at a strength of 0·3 per cent., cannot always be relied upon to cure animals of scab, and should, in the interests of safety, be avoided at all stages in the process of dipping.

REFERENCES.

GREEN, H. H. (1915). "The Sulphur Sheep Dips." *3rd and 4th Repts. Dir. Vet. Res., Un. S. Afr.*, pp. 140-142.

GREEN, H. H. (1919). "Dips and Dipping." *Industries Bulletin No. 37*, pp. 1-28; No. 37A.

Researches into Dips and Dipping. *

A. Lime-Sulphur Dips.

VI. A Survey of the Behaviour of Lime-Sulphur Dips under Field Conditions. †

By

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CONTENTS.

I. INTRODUCTION.

As has been pointed out in Paper A.I of the series, a field study of lime-sulphur dips was undertaken in order to answer various practical questions which had arisen from time to time. Such questions include the rôle played by various qualities of commercial lime samples used in the preparation of home-made lime-sulphur concentrates, the influence of farm water in its dilution, the liability to oxidation of lime-sulphur solutions on standing, the effect of dipping on such solutions, e.g. the diminution in strength after passing a known number of animals through a bath of known initial concentration and volume, etc. An adequate answer to such and other questions would be of definite assistance to field veterinarians, farmers and dip inspectors.

In the course of this study about 160 full analyses of field dips and concentrates have been made, the method of analysis employed being the volumetric cadmium acetate method as described in Paper II of this series. The lowest concentration of polysulphide shown by the home-made concentrates was 9·6 gm. polysulphide sulphur per 100 c.c. concentrate, the highest concentration being 30·4 gm. The minimum strength of the diluted dips or dipwashes before and after dipping was 0·43 and 0·16 gm. sulphide sulphur per 100 c.c. dipwash respectively. In Table I average figures have been compiled for 84 field dips, all analyses for dips showing faulty sampling or being otherwise doubtful, having been discarded.

TABLE I.

Field Dipping Strengths.

P.S.S. limits (gm. S. per 100 c.c. dipwash).	Before dipping.		After dipping.	
	No. of samples.	Average.	No. of samples.	Average.
Between 0·00 and 0·20.....	0	—	1	0·16
„ 0·20 and 0·40.....	0	—	6	0·33
„ 0·40 and 0·60.....	2	0·47	9	0·51
„ 0·60 and 0·80.....	2	0·66	10	0·71
„ 0·80 and 1·00.....	11	0·92	5	0·86
„ 1·00 and 1·20.....	3	1·15	6	1·14
„ 1·20 and 1·40.....	5	1·32	5	1·31
„ 1·40 and 1·60.....	7	1·46	1	1·54
„ 1·60 and 1·80.....	6	1·72	2	1·67
„ 1·80 and 2·00.....	2	1·90	0	—
„ 2·00 and 2·20.....	1	2·15	0	—
AVERAGE.............	39	1·26	45	0·81

P.S.S. = Polysulphide sulphur.

Under the present regulations of the Department of Agriculture, as recorded in Paper I of this series, the specified initial concentration of lime-sulphur dipwashes is laid down to be 1·5 gm. sulphide sulphur per 100 c.c. dipwash. From the above table about 75 per cent. of the dipwashes examined fall below this specified strength, although the

true average (1·26) of all samples examined does not fall very much below this limit. The average concentration of the washes after dipping is shown to be 0·8, a figure Green (1915) accepted as still adequately effective against scab. On the other hand, in some samples the concentration does fall as low as 0·47 for the fresh wash, and even as low as 0·33 and 0·16 in the case of washes after dipping. If the departmental figure of 1·5 were anywhere near the minimum effective limit, this state of affairs would naturally cause grave concern. However, from the experiments reported in the previous paper (Paper V), a dip of strength 0·33 might still be effective. Of all the dips examined only one falls below this minimum effective limit, which means that the last batch of sheep to pass through this particular wash was not effectively cured of scab if infected.

What is important, however, is the wide range of variations revealed by Table I. In other words, Table I shows how necessary it is to use a considerable safety factor by fixing the recommended dipping strength appreciably above the minimum effective limit in order to make all dipping effective. Even then this safety factor reflects only the better half of the true field picture, since all field work for the present study was done by specially selected men. To this must further be added the effect of the psychological factor, in as much as all field work done for this investigation was to be controlled in the laboratory, whereas under normal field practice no such direct control is anticipated. On the other hand, in the interests of economy, and from the point of view of possible damage to the wool, a lowering of the recommended strength of 1 5 would be highly desirable, especially in view of the observation that concentrations as low as 0·3 may still be effective. It is clear, however, that such steps will be justified only if a greater measure of control can be exercised, with a subsequent lowering of the deviation from the recommended strength.

If, for the purpose of this study, we leave the actual analytical control of samples taken from time to time during the course of dipping out of consideration, the all important question to be answered is in how far the various factors operating during the process of dipping are capable of control or manipulation under field conditions. Obviously, it would be of considerable practical importance if the factors concerned can be so controlled, that it would become possible to tell farmers and dip inspectors to what extent the strength of a bath of known volume and initial concentration would diminish after a certain number of animals had passed through. On the other hand, this diminution in strength per animal may, as a result of factors over which we have little or no control, vary so greatly from one set of conditions to another, that its practical usefulness may be zero.

II. THE PREPARATION OF THE CONCENTRATE.

In the preparation of home-made concentrates, the boiling up of the lime and sulphur in an aqueous medium results in the action of the free sulphur on the active lime with the formation of the polysulphides of calcium and calcium thiosulphate. For determining

the chemical reactions involved, a definite weight of different lime samples was boiled up with excess flowers of sulphur, the solution made up to a definite volume and then analysed. The results obtained have been tabulated in Table II.

TABLE II.
Reaction of Lime with Sulphur.

Lime sample.	Dipping Coeff. II of lime % $Ca(OH)_2$.	M.S.S. Eq. per 2·5 gm. lime (gm.S).	Thiosulphate per 2·5 gm. lime (gm.S).	x in CaS_x.	Ratio Thios. S/M.S.S. Eq.
K. 12	19·9	0·135	0·147	5·37	1·08
K. 3	37·5	0·265	0·280	4·83	1·06
K. 9	48·9	0·356	0·344	4·87	0·97
K. 10	61·2	0·433	0·464	4·98	1·07
K. 4	66·0	0·495	0·500	4·72	1·01
K. 11	67·2	0·495	0·480	4·42	0·97
F. 6	67·8	0·485	0·495	5·11	1·02
K. 7	75·3	0·545	0·540	4·78	0·99
K. 5	76·5	0·545	0·550	4·78	1·01
Tvl. 5	76·8	0·549	0·560	5·12	1·02
K. 6	77·1	0·545	0·540	4·66	0·99
TKi. 1	80·6	0·585	0·590	4·80	1·01
F. 2	82·8	0·595	0·560	4·73	0·96
Tvl. 7	84·4	0·610	0·605	4·62	0·99
K. 1	84·5	0·600	0·610	4·93	1·02
Tvl. 12	86·1	0·634	0·592	5·37	0·93

M.S.S.Eq. = Monosulphide sulphur equivalent.

For the polysulphides formed the atomic ratio of calcium to sulphur was found to be over 5·0 in at least four cases, so that, in accordance with the findings of Patel, Sen-Gupta and Chakravarti (1930) for organic polysulphides, an inorganic polysulphide of the order CaS_6 or higher must be present. What other polysulphides are present, and how they are distributed to make up the average value of $CaS_{4·6}$ to $CaS_{4·8}$ we shall not endeavour to discuss here. It would suffice to state that the higher polysulphides in solution are extremely unstable, and that it is quite possible for an initial solution represented by $CaS_{5·0}$ to depreciate to $CaS_{4·8}$ by the time it is analysed, unless all due precautions are taken. It is possibly for this very reason that all proprietary concentrates and home-made dips never show a composition appreciably above $CaS_{4·6}$.

However, what is of importance as far as the reactions involved are concerned, is the observation that the ratio of thiosulphate sulphur to monosulphide sulphur equivalent is unity, as shown in the last column of Table II. Hence, for every atom of sulphur going into solution as monosulphide sulphur equivalent, there is formed half a molecule of thiosulphate; or, what amounts to the same, for every two atoms of calcium attached to sulphur to form polysulphides there is used one atom of calcium for the formation of calcium thiosulphate. Taking, as an example, the formation of the tetrasulphide from calcium hydroxide and sulphur, the equation for the reaction may be represented as follows:

$$3 \ Ca(OH)_2 + 10S \rightarrow 2 \ CaS_4 + CaS_2O_3 + 3 \ H_2O.$$

Similarly, for the pentasulphide, the equation becomes:—

$$3 \ Ca(OH)_2 + 12S \rightarrow 2 \ CaS_5 + CaS_2O_3 + 3 \ H_2O.$$
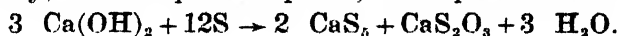
In the case of proprietary preparations and also in the case of home-made concentrates in the field, the thiosulphate content may be appreciably lower than these equations demand. This, however, is due to the relative insolubility of calcium thiosulphate in concentrated solutions of calcium polysulphide, and its subsequent removal with the sediment in the process of clarifying the concentrate.

If now we assume that the directions for the preparation of the concentrate are too simple to allow of faulty preparation, any fluctuation in the quality of concentrate obtained can only be ascribed to the impurity of the ingredients used. The sulphur is excluded from the considerations under review since flowers of sulphur of a high quality is generally used. Considering the relative quantities of lime and water used, the effect of any impurities in the water cannot be expected to be appreciable. As an experimental verification of this conclusion the following data * have been collected in Table III.

(a) EFFECT OF WATER ON CONCENTRATE.

TABLE III.

Effect of Water on Concentrate.

Lime and water sample.	Origin of water.	Dipping coefficient with this water.	Dipping coefficient with Onderstepoort water.
1..............	Beaufort West........	22·1 % CaO	22·7 % CaO
2..............	,, ...	47·1 % ,,	46·0 % ,,
3..............	,, ...	39·7 % ,,	39·7 % ,,
4..............	Gordonia.............	34·0 % ,,	37·0 % ,,
5..............	,, ...	26·6 % ,,	27·3 % ,,
6..............	Williston.............	42·0 % ,,	42·0 % ,,
7..............	Carnarvon.....	38·0 % ,,	36·0 % ,,
8..............	Van Wyksvlei....... ..	38·0 % ,,	36·0 % ,,
9..............	Calvinia.............	19·1 % ,,	19·1 % ,,
10..............	Concordia..	38·4 % ,,	38·4 % ,,
11..............	,, ...	17·9 % ,,	18·3 % ,,
12..............	Namaqualand.........	36·0 % ,,	36·0 % ,,

If the above table shows that the effect of the water used in boiling up the concentrate is negligible, it also shows that the limes used vary greatly in purity. For the purpose of the present investigation 19 lime samples, used in the field for preparing home-made lime-sulphur concentrates, have been studied in the laboratory. Besides the determination of the sulphate sulphur, total calcium and free calcium hydroxide content of these limes, their " dipping coefficients " have also been determined by allowing a known quantity to react with excess sulphur at boiling point. 2·5 gm. lime were mixed with ca. 10 gm. flowers of sulphur and boiled with water for 40 minutes, immediately afterwards filtering off into a 500 c.c. measuring flask, washing, and filling up to volume in the cold. By analysing the thus obtained lime-sulphur solution, two dipping

*Data from a departmental report, 1931.

coefficients were determined, once by expressing the total calcium found in the solution as the percentage $Ca(OH)_2$ of the total lime used—Coefficient I—and secondly, calculating the $Ca(OH)_2$ equivalent of the sulphur in solution as calcium polysulphides and calcium thiosulphate, and expressing this $Ca(OH)_2$ content as a percentage of the total lime used—Coefficient II. In Table III these results have been tabulated in ascending order of lime purity. The figures given under Coefficient I. will of course rise above the true values when the lime contains other soluble calcium salts, as for example calcium chloride, and to some extent calcium sulphate. This explains why the figures under Coefficient I are always slightly above those under Coefficient II. On the other hand, any free alkali or earth alkali other than calcium oxide and/or calcium hydroxide present in the lime will result in Coefficient II. falling out too high.

(b) PURITY OF LIME SAMPLES.

TABLE IV.

Purity of Lime Samples.

Lime sample.	Origin and Brand of Lime.	% SO_4 sulphur in lime.	% Ca in lime.	% Active or free $Ca(OH)_2$	Dip coeff. I.	Dip coeff. II.
K. 12	Not known..................	0·16	38·2	—	23·8	19·9
K. 3	Native Reserve, Rooifontein, Namaqualand..............	0·20	38·6	34·8	37·7	37·5
Tvl. 1	MacKenzie Siding—local......	0·52	40·6	36·7	41·0	39·1
K. 9	Piquetberg and Bredasdorp Lime Works..............	0·70	36·6	49·9	51·7	48·9
K. 1 and 2 Sp.	„ „	0·37	48·7	—	53·2	52·3
K. 10	„ „	0·70	42·1	62·5	61·6	61·2
K. 11	Taungs Afric. Lime Works Afric. Brd.................	0·38	45·1	—	67·8	67·2
K. 4	Namaqualand—local.........	—	42·1	—	68·2	66·0
F. 6	White Lime, Ltd., Johannesburg....................	—	—	—	68·7	67·8
K. 7	Taungs.....................	—	—	—	76·0	75·3
Tvl. 5	Not known..................	1·06	44·7	78·1	78·2	76·8
K. 5	Northern Lime Co., Taungs...	0·33	46·8	—	78·3	76·5
K. 6	White Lime, Ltd., Johannesburg....................	0·70	46·3	—	78·4	77·1
TKi. 1	Taungs Afric. Lime ,Works, Afric. Brd.................	0·27	50·2	—	83·8	80·6
F. 2	Buxton Lime Works, Taungs..	0·37	50·8	—	84·5	82·8
K. 1	Northern Lime Co., Taungs...	0·42	48·6	—	84·9	84·5
Tvl. 7	Not known..................	0·28	48·4	—	85·2	84·4
Tvl. 12	Uitloop—Potgietersrust District	0·15	49·5	88·6	86·4	86·1
Tvl. 11	„ „	0·56	49·8	84·1	90·3	87·8
Lab. sample	E. Merck, Darmstadt........	trace	49·4	92·3	91·8	90·1

From the table it will be seen that the limes used in the preparation of home-made concentrates vary in purity from 20 per cent. to 90 per cent., though the higher purity samples frequently include limes only partially slaked or hydrated. But even a lime of purity approximately 20 per cent. should still yield a dipwash of 0·6 to

0·7 per cent. polysulphide sulphur when prepared according to standard specification. However, in considering this matter from the point of view of field practice, there is at least one important factor to be borne in mind, and this is the factor of field efficiency. By this is meant the efficiency with which the official specifications can be carried out under actual field conditions, taking into account all possible contributing factors. These contributing factors are various, and range from the mere mechanical measurement of quantities to the uncontrollable chemical effect of the water used for the dilution of the concentrate. As regards field efficiency, Green (1915) as early as 1915 pointed out that field samples invariably fail to come up to the strength attainable in the laboratory with the same lime; that is, that the field efficiency apparently invariably falls out below 100 per cent. What this field efficiency actually amounts to under South African conditions we shall attempt to deduce from the data now available.

In trying to determine this efficiency, the amounts of sulphur brought into solution when operating under optimum conditions in the laboratory, must be compared with the amounts brought into solution (in diluted dip) under actual field conditions, using the same quantity of the same lime. As all results for the laboratory work in the determination of the dipping coefficients of the various lime samples were calculated on the basis of the amount of polysulphide sulphur brought into solution by 2·500 gm. lime, the analyses of the diluted dip samples (before dipping) obtained from the field were all recalculated to this basis of 2·500 gm. lime. For this conversion the simple formula $F = 25.x \times \frac{1}{a}$ applies, where:—

F is the amount of polysulphide sulphur (in gm. in the diluted dip) brought into solution by 2·500 gm. lime under field conditions;

x is the concentration of the diluted field dip in gm. polysulphide sulphur per 100 c.c. solution; and

a is the weight of lime (in lb.) used per 100 gallons of diluted dip.

The comparison on a purely polysulphide sulphur basis is here necessary, since this is apparently the chief ingredient of a lime-sulphur dip actually responsible for its effectivity against scab, whether this be directly or indirectly. The results thus obtained have been tabulated in Table V.

TABLE V.

Field Efficiency.

Dip.		Dipping coeff. II.	Lb. lime/ 100 gals. diluted dip.	Conc. of concentrate. %.	Polysulphide in dil. dip. (gm. S/100 c.c.).	x in CaSₓ.	Field efficiency.
							%
K. 12	Lab. sam. Field ,,	19·9	19·0	12·4	0·725 0·573	5·37 4·05	79·0
K. 3	Lab. sam. Field ,,	37·5	25·0	17·4	1·279 0·971	4·83 4·78	75·9
K. 9	Lab. sam. Field ,,	48·9	22·0	17·1	1·733 1·316	4·87 4·71	75·9
K. 10	Lab. sam. Field ,,	61·2	20·0	26·2	2·156 1·545	4·50 4·21	71·6
K. 4	Lab. sam. Field ,,	66·0	30·0	30·4	2·335 1·581	4·72 4·04	67·8
K. 11	Lab. sam. Field ,,	67·2	19·0	27·1	2·185 2·130	4·42 4·21	97·5
F. 6	Lab. sam. Field ,,	67·8	23·0	14·9	2·480 1·871	5·11 4·79	75·4
K. 7	Lab. sam. Field ,,	75·3	20·0	22·3	2·605 1·240	4·78 3·45	47·5
K. 5	Lab. sam. Field ,,	76·5	20·0	24·1	2·605 1·591	4·78 4·34	61·0
Tvl. 5	Lab. sam. Field ,,	76·8	20·0	21·9	2·808 2·360	5·12 4·74	84·0
K. 6	Lab. sam. Field ,,	77·1	20·0	23·1	2·540 1·794	4·66 3·78	70·6
TKi. 1	Lab. sam. Field ,,	80·6	20·0	16·2	2·805 2·038	4·80 4·06	72·6
F. 2	Lab. sam. Field ,,	82·8	20·0	9·6	2·815 2·205	4·73 4·22	78·3
Tvl. 7	Lab. sam. Field ,,	84·4	20·0	22·0	2·820 1·803	4·62 4·68	63·9
K. 1	Lab. sam. Field ,,	84·5	20·0	27·3	2·960 1·899	4·93 3·87	64·2
Tvl. 12	Lab. sam. Field ,,	86·1	20·0	20·0	3·408 2·096	5·12 3·90	61·5

The minimum field efficiency found was 47·5 per cent., the maximum efficiency closely approximating 100 per cent. However, on the whole the normal field efficiency seems to lie around 70 per cent., the average of all the values tabulated being 71·7 per cent. Considering the possibility of a very appreciable fraction of this 30 per cent. loss in efficiency being caused by the effect of the dilution water on the polysulphides in solution, an all-round efficiency of 70 per cent. is certainly very satisfactory.

(c) DILUTION OF THE CONCENTRATE.

In order to study the possible effect of the water in the dilution of the concentrate, some of the waters received from the field were subjected to partial analysis in the laboratory. The analyses for non-volatiles or total solids, ash, sulphate sulphur, and total calcium have been tabulated in Table VI.

TABLE VI.

Water Analysis.

Water.	District.	Farm.	Solids (mgm/ 100 c.c.).	Ash (mgm/ 100 c.c.).	SO$_3$ (mgmS/ 100 c.c.).	Ca (mgm/ 100 c.c.).
Tvl. 5	Ermelo.........	Wyntoun........	8·2	2·6	0·0	0·0
Tvl. 1	Pretoria........	La Rochelle No. 610	17·4	8·2	0·0	0·0
K. 6	Gordonia.......	Swemkuil........	19·8	13·2	1·3	2·4
K. 9	Sutherland......	Agterplaas.......	42·0	17·2	2·0	5·1
K. 10	Sutherland......	Jakhalsfontein....	48·4	24·4	3·1	6·7
Tvl. 12	Pietersburg.....	Klipdam........	59·2	42·6	1·9	1·7
Tvl. 11	Pietersburg.....	Klipdam.........	70·2	54·4	4·0	4·8
Tvl. 6	Ermelo.........	Tafelkou No. 36..	184	136	23·2	18·4
K. 1	Calvinia........	Taaiboshoek.....	313	126	17·3	23·7
K. 11	Jansenville......	Soutpansnek.....	400	301	36·2	26·5
K. 12	Jansenville......	Soutpansnek.....	410	312	39·0	26·8
K. 2	Van Rhynsdorp.	Vredendal.......	444	217	35·6	14·0
K. 5	Kenhardt.......	Mariasput........	478	252	131	37·5
K. 3	Namaqualand ..	Soutvlei...	530	383	44·7	35·1
K. 4	Namaqualand...	Holgat......... .	640	414	60·1	22·9
K. 1 & 2 Sp.	Calvinia........	Downes..........	662	347	38·2	60·0
K. 7	Carnarvon......	Eendefontein.....	—	—	7·1	5·1
Tvl. 7	Belfast..... ...	Houtenbek.......	—	—	0·0	1·9
TKi. 1	Cathcart........	Rockford.......	—	—	0·0	1·7
N. 7	Greytown.......	Umvoti Location	—	—	0·0	0·0
N. 3	Umzinto........	Farm No. L/A477	—	—	—	3·4
TKi. 3	Kingwilliams-town	Farm No. 309....	— -	—	—	2·3
TKi. 4	Kingwilliams-town	Nonibe Location..	—	— -	—	10·4
F. 2	Barkly West....	Witfontein.......	—	— -	—	6·8

In every case the water sample was filtered before analysis. In spite of this the total solids in solution were found to rise as high as 0·6 gm. per 100 c.c. and higher, while the maximum ash value exceeded 0·4 gm. per 100 c.c. The sulphate sulphur fluctuated between zero and 0·13 gm. SO$_3$ per 100 c.c., while the total calcium fluctuated between zero and 0·06 gm. per 100 c.c. Some of the waters contain relatively large concentrations of magnesium and sodium sulphate, while others again apparently contain appreciable concentrations of calcium bicarbonate, since the total calcium exceeds by far the sulphate sulphur necessary for the formation of calcium sulphate. It may thus be expected that, in the case of some of the waters at least, an appreciable reaction between the constituents of the water and the calcium polysulphide will set in, resulting in the decomposition of some of the polysulphide.

To illustrate some of the more important reactions concerned, a few typical water samples were selected, and their effect upon the polysulphides of calcium studied experimentally. For this purpose 5 c.c. of a proprietary concentrate was diluted to 250 c.c. with the water concerned, atmospheric oxygen excluded, and the diluted dip

allowed to stand until complete sedimentation of the precipitate occurred. The perfectly clear solutions were then analysed. These were compared with similar dilutions made by using distilled water. On observing the visible changes, the following was noticed :—

Water sample.	Characteristics.	Sedimentation.
K. 1...................	High in solids and ash; high in sulphate and Ca; high in bicarbonate	Appreciable.
K. 5...................	High in solids and ash; high in Na and Mg sulphate; high in calcium	Fairly appreciable.
Tvl. 1.................	Low in solids and ash; no sulphate and no calcium	Slight.
Onderstepoort water.....	High in calcium; high in bicarbonate	Fairly appreciable.
Distilled water..........	Pure..............................	None.

The chemical analyses have been collected in Table VII.

TABLE VII.

Effect of Dilution Water.

Dip analysis.	Distilled water.	Tvl. 1 water.	K. 1 water.	K. 5 water.
M.S.S.E. (gm./100 c.c.)........	0·157	0·157	0·157	0·157
Thiosulphate S. (gm./100 c.c.)...	0·012	0·012	0·012	0·012
Total S found (gm./100 c.c.)....	0·776	0·778	0·720	0·794
S in water (gm./100 c.c.).......	0·000	0·000	0·007	0·052
.˙. Total dip S...............	0·776 ·	0·778	0·713	0·742
.˙. Per cent. S lost...........	0·0	0·0	8·2	4·5
Total Ca found (gm./100 c.c.)...	0·206	0·206	0·202	0·246
Ca in water (gm./100 c.c.)......	0·000	0·000	0·024	0·038
.˙. Total dip Ca..............	0·206	0·206	0·178	0·208
.˙. Per cent. Ca lost..........	0·0	0·0	14·0	0·0

These results show that the dilution water apparently has no influence on the thiosulphate and monosulphide sulphur equivalent content. On the other hand, both calcium and polysulphide sulphur may be precipitated.

The results obtained in the laboratory have also been substantiated by field samples. In the following table—Table VIII—the results on a number of proprietary concentrates diluted one in twenty-five have been recorded. A sample of the same concentrate was diluted to the same extent in the laboratory with distilled water, and the analysis compared with that of the diluted dip received from the field. Only proprietary concentrates were considered, since the volume of home-made concentrates cannot be relied on.

TABLE VIII.

Effect of Water in Field Dilution.

Dip.		Polysulphide (gm. S/100 c.c.).	x in CaS$_x$.	Per cent. loss on dilution in field.
TKi. 7	Lab. sample....	1·456	4·45	
	Field ,, ...	1·406	4·16	} 3
TKi. 9	Lab. sample............	1·452	4·54	
	Field ,, ...	1·279	4·50	} 12
TKi. 4	Lab. sample............	1·528	4·66	
	Field	1·179	4·46	} 23
TKi. 8	Lab. sample............	1·447	4·55	
	Field ,, ...	1·105	4·44	} 24
N. 3	Lab. sample............	1·016	4·68	
	Field ,, ...	0·822	4·70	} 26
N. 11	Lab. sample............	0·939	4·58	
	Field ,, ...	0·672	4·58	} 28
N. 9	Lab. sample............	0·955	4·63	
	Field	0·647	4·49	} 32

Here again there is a distinct loss on dilution, due to the reaction of the water, some of the higher polysulphides being decomposed with a resultant fall in the atomic ratio of calcium to sulphur. If we accept the field measurements to be correct, that is if the dilution in the field was actually one in twenty-five, then the loss on dilution of up to 30 per cent. would appear to account for the loss in field efficiency of 30 per cent. as recorded in Table V. This loss is, of course, incapable of control, since the field worker cannot normally obviate the effect of the impurities in the water except in cases where rain water is available.

III. THE EFFECT OF DIPPING ON THE TANK FLUID.

During the process of dipping various foreign substances are brought into intimate contact with the constituents of the dipping bath. In the order of their influence on the composition of the bath they may be arranged as the influence of atmospheric and loosely-bound oxygen and carbon dioxide, the effect of suint constituents going into solution, and the adulteration with dirt, animal exrements, and earth. Owing to the air occluded in the fleece and the intensive mechanical agitation of the bath accompanying dipping, large volumes of air are brought into intimate contact with the dip, and accordingly appreciable oxidation and carbon dioxide disintegration may come into play. This atmospheric effect we shall discuss more closely in a following paper.

The dipping of wool-bearing animals, however, also results in an appreciable washing-out of the suint of the wool, these suint constituents effecting the composition of the dip in various ways. The alkali carbonate of the suint will react precipitating the calcium and forming alkali polysulphides.

$$CaS_4 + K_2CO_3 \rightarrow CaCO_3 + K_2S_4$$

The alkali soaps in the suint will precipitate the calcium as calcium soaps, again forming the alkali polysulphides. On the other hand, the entering of certain suint constituents into solution may improve the dip in so far as its surface tension towards the wool and the skin may be diminished, thereby increasing its wetting power and consequently its efficacy. In fact, this phenomenon is claimed to have been actually observed in field practice. This is a most important observation from a practical point of view, and we hope to be able to study it more closely in the near future. As far as the present paper is concerned we shall confine ourselves to a study of the collective effect of dipping on the composition of the dip in the field.

In the process of dipping it is found that the polysulphide sulphur in solution decreases, and the thiosulphate increases as has been shown by Van Zyl (1926). Here two questions chiefly deserve consideration, viz. the manner in which the polysulphide decreases, and the manner in which the thiosulphate increases at the expense of the polysulphide. For the sake of simplicity we shall consider the formation of the thiosulphate first.

In proceeding to investigate the manner in which the thiosulphate increases at the expense of the polysulphide, and also to consider such factors as initial polysulphide concentration, bath volume, and the number of animals dipped, it must be remembered that in the process of dipping there is, in addition to a progressive decrease in polysulphide and increase in thiosulphate concentration, also a progressive loss in dipwash, as each animal carries with it a certain volume of dip on emerging from the bath. Naturally, the volume of the dip, thus lost mechanically, will depend on the size of the animal, the weight and nature of its fleece, the manner of dipping, and the design and construction of the dipping outfit. However, to take due account of all these factors would make a field study well-nigh impossible. The only practicable solution here seems to be to resort to averages.

(a) Dipwash Removed during Dipping.

In several cases fairly accurate information was obtained regarding the quantity of dip carried off on dipping a certain known number of animals. In the following table (Table IX) the results of dipping close on to 12,000 animals have been recorded.

TABLE IX.

Dip Lost on Dipping.

	Number of animals dipped.	Initial volume of bath (gals.).	Final volume of bath (gals.).	Volume lost on dipping (gals.).	Volume lost per animal (gals.).
Case 1...........	562	455	325	130	0·23
Case 2...........	197	390	535	45	0·23
Case 3...........	560	395	265	130	0·23
Case 4...........	737	520	325	200	0·27
Case 5...........	536	390	225	165	0·31
Case 6...........	536	485	275	210	0·39
Case 7...........	900	800	350	450	0·50
Case 8...........	805	700	275	425	0·53
Case 9...........	3,813	2,500	475	2,025	0·53
Case 10...........	982	1,300	600	700	0·71
Case 11...........	822	1,600	650	950	1·15
Case 12...........	1,262	1,900	400	1,500	1·19
TOTAL........	11,712	---	—	6,930	0·59

From the 11,712 animals dipped the average mechanical loss of dip is found to be 0·59 gallons per animal, the minimum and maximum limits being 0·23 and 1·19 gallons respectively.

(b) THE EFFECT OF DIPPING ON THIOSULPHATE CONTENT.

For every animal immersed into the bath a certain quantity of thiosulphate is formed. On leaving the bath the animal, however, carries off with it a certain quantity of thiosulphate, depending on the concentration of thiosulphate in the bath at that moment. When the second animal enters, it enters a bath somewhat smaller in volume, but with a somewhat higher thiosulphate and somewhat lower polysulphide concentration. For the rest it repeats the process executed by the former animal. If, now, the amount of thiosulphate formed per animal varies, in the first instance, with the nature of the animal which in turn is a function of various unknown variables, and in the second instance with the composition of the bath at the time of immersion, the process, considered mathematically, becomes highly involved. However, taking into consideration the various limits placed on the accuracy of all field measurements, and also the various unknown factors influencing dipping such as nature and weight of fleece, design of dipping plant, etc., it would be of little use to stress unduly the strict mathematical treatment of the problem.

What is actually measured is the initial concentration of the thiosulphate in a bath of known volume, and the final concentration of thiosulphate in the wash remaining in the tank, the volume of which is also measured or known. The simplest method of approach here is to consider the quantity of thiosulphate mechanically removed from the bath during the process of dipping; for obviously, once we know the quantity removed mechanically, the initial quantity and the final quantity, the calculation of the quantity formed chemically

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is a simple matter. Given a definite set of dipping conditions under which a known number of animals are dipped, the concentration of thiosulphate in the dipwash mechanically removed from the bath, will rise progressively from a concentration practically equal to the initial concentration, to a concentration practically equal to the final concentration. If, therefore, the initial concentration is T_1 and the final concentration T_n, the average concentration may, for all practical purposes, be taken to be:—

$$T_a = \frac{(T_1 + T_n)}{2}$$

If these concentrations be expressed in gm. S. per 100 c.c., and if the initial volume of the bath be V_1, decilitres and the final volume V_n decilitres, the amount of thiosulphate, expressed in gm. sulphur, mechanically removed from the bath may be expressed by:—

$$\sigma = \frac{(T_1 + T_n)}{2} \times (V_1 - V_n) \text{ gm. sulphur.}$$

If n animals are dipped then the amount of thiosulphate removed mechanically per animal will be:—

$$\delta\sigma = \frac{(T_1 + T_n)}{2} \times \frac{(V_1 - V_n)}{n} \text{ gm. sulphur.}$$

Expressing the volume V_1 and V_n in gallons, this equation becomes:—

$$\delta\sigma = 10 \times 4.54 \times \frac{(T_1 + T_n)}{2} \times \frac{(V_1 - V_n)}{n}$$

$$= 22.7 (T_1 + T_a) \times \frac{(V_1 - V_n)}{n} \text{ gm. sulphur.}$$

Using this method of calculation, and considering in the first instance only those dips where the final dip volume after dipping was definitely known, Table X was compiled.

TABLE X.

Effect of Dipping on Thiosulphate Content.

Dip.	Initial Thios. conc. (gm.S/ 100 c.c.).	Final Thios. conc. (gm.S/ 100 c.c.).	Initial volume (gals.).	Final volume (gals.).	Initial Thios. in bath (gm.S).	Final Thios. in bath (gm.S).	Thios. lost (gm.S).	Number animals dipped.	Thios. formed per animal (gm.S).
N. 7........	0·150	0·165	395	265	2,690	1,986	933	560	0·4
TKi. 7......	0·018	0·083	520	325	425	1,225	463	737	1·7
K. 7........	0·141	0·384	700	275	4,481	4,794	5,057	805	6·7
Tvl. 6......	0·243	0·621	2,500	475	27,580	13,400	39,730	3,813	6·7
K. 10.......	0·282	0·588	800	350	9,517	9,346	8,684	900	9·5
K. 1 and 2 Sp.	0·115	0·416	1,900	400	9,922	7,556	18,110	1,262	12·5
TKi. 1......	0·352	0·640	1,300	600	20,770	17,440	15,770	982	12·6

The results obtained for the quantities of thiosulphate formed per animal under different sets of dipping conditions are somewhat unexpected. It could hardly have been expected that as much as 12·6 gm. thiosulphate sulphur would be formed by dipping a single animal; on the other hand, the great fluctuation from 0·4 to 12·6 gm. thiosulphate sulphur per animal indicates the variety of factors operating under different conditions of dipping.

To serve as a further check on these results, use has been made of the average and minimum loss in gallons of dip per animal, as given in Table IX, to determine analogous figures for those dips where the actual measurement of the final volume in the tank at the end of dipping was not available. These results have been tabulated in Table XI. The figures in column 6 were determined by taking the volume of dip removed per animal equal to the average figure of 0·59 gallons; the figures in column 7 were obtained by employing the minimum loss of 0·23 gallons per animal, and as such represent the maximum amount of thiosulphate formed per animal.

TABLE XI.

Dip.	Initial Thios. conc. (gm. S/100 c.c.).	Final Thios. conc. (gm. S/100 c.c.).	Initial volume (gals.).	Number animals dipped.	Thios. formed per animal (0·59 basis). (gm. S.)	Thios. formed per animal (0·23 basis). (gm. S.)
K. 9	0·211	0·358	600	2,181	—	1·1
TKi. 4.....	0·075	0·225	500	1,600	—	1·3
TKi. 5.....	0·012	0·096	400	775		1·5
N. 12......	0·095	0·144	500	549	1·4	1·8
N. 9.......	0·135	0·201	390	458	1·7	2·2
K. 2.......	0·089	0·143	600	508	2·1	2·6
N. 3.......	0·111	0·128	625	160	2·3	2·9
K. 3.......	0·141	0·326	1,700	2,739	3·1	4·2
Tvl. 7......	0·270	0·450	950	1,487	3·2	4·3
K. 6.......	0·230	0·473	800	1,444	—	4·9
K. 5.......	0·230	0·409	600	723	4·3	5·8
F. 2.......	0·360	0·540	800	600	8·4	9·9

On the whole this table substantiates the results recorded in Table X, the maximum amount of thiosulphate here formed being represented by 9·9 gm. sulphur. It is significant that the figures recorded in columns 6 and 7 do not differ to any great extent. This means that the effect of the final volume on the figures calculated for the amount of thiosulphate formed per animal is small, so that the normal inaccuracies inherent in all field measurements cannot influence the thiosulphate values in Tables X and XI to any appreciable extent. This implies that the wide fluctuations in the quantities of thiosulphate formed per animal under different conditions, cannot be ascribed to any normal inaccuracy in field measurement.

(c) Effect of Dipping on Polysulphide.

The effect of dipping on the polysulphides of calcium in solution can conceivably be twofold; (a) in reducing the total amount of polysulphide sulphur in solution, and (b) in changing the nature of the polysulphides in solution.

If the higher polysulphides are less stable than the lower members of the group, obviously these higher polysulphides will be decomposed at a much greater rate, with a resulting fall in the atomic ratio of calcium to sulphur. The results in Table XII have been collected to show that this change in the nature of the polysulphides actually occurs as a result of dipping.

TABLE XII.

Effect of Dipping on Nature of Polysulphide.

Dip.	Polysulphide concentration *before* dipping (gm. S/100 c.c.).	X in M_2S_x before dipping.	Polysulphide concentration *after* dipping (gm. S/100 c.c.).	X in M_2S_x after dipping.
Group A.				
TKi. 4.	1·18	4·46	0·46	1·34
TKi. 5.	0·89	4·46	0·44	1·45
K. 12.	0·43	4·05	0·16	1·50
K. 3.	0·97	4·78	0·25	1·70
TKi. 3.	2·15	4·62	0·36	1·71
N. 9.	0·65	4·49	0·30	1·93
K. 9.	1·16	4·71	0·32	1·94
K. 2.	0·99	4·61	0·38	2·20
TKi. 7.	1·41	4·16	0·65	2·20
K. 7.	0·99	3·45	0·76	2·38
K. 1.	1·52	3·87	0·60	2·57
K. 10.	1·24	4·21	0·68	2·58
K. 6.	1·43	3·78	0·81	3·00
K. 5.	1·27	4·34	0·55	3·12
TKi. 8.	1·10	4·44	0·95	3·29
F. 2.	1·76	4·22	1·16	3·45
K. 11.	1·60	4·21	1·16	3·90
TKi. 1.	1·63	4·06	1·39	3·97
Tvl. 7.	1·44	4·68	0·85	4·04
N. 12.	0·84	4·66	0·53	4·20
N. 3.	0·82	4·70	0·77	4·38
Group B.				
TKi. 3.	0·95	—	0·44	1·83
TKi. 4.	0·52	2·07	0·52	2·03
K. 2.	0·99	4·61	0·38	2·17
TKi. 7.	0·84	2·35	0·46	2·22
TKi. 8.	1·11	3·66	0·65	2·41
K. 1.	—	—	0·65	2·46
K. 7.	1·30	3·54	0·80	2·60
N. 9.	1·38	4·52	0·85	3·29
TKi. 5.	1·42	4·40	0·73	4·01

The results under Group A represent dips which had been freshly prepared before dipping; the results under Group B represent dips which had been previously used for dipping, but which had been re-strengthened for the occasion by adding fresh concentrate to the

bath. The table shows clearly that there is both a decrease in the polysulphide sulphur concentration and a marked change in the nature of the polysulphide. The greatest change in the nature of the polysulphides is shown by dip TKi.4 where the ratio of alkali metal to sulphur had fallen as low as $1 \cdot 34$. This depreciation in the order of the polysulphides in solution is of course dependent on various factors such as initial polysulphide concentration, the volume of the bath, the number of animals dipped, etc. However, from the wool-producer's point of view this change is most important, and must be studied very closely. There can be little doubt that a polysulphide solution represented by $CaS_{1.3}$—which by its very nature must contain an appreciable concentration of HS ions due to hydrolysis—must, provided the concentration is high enough, be far more injurious to the wool fibre than a polysulphide solution represented by $CaS_{4.2}$. In this connection a comparison of dip TKi.4 with dip N.12 (after dipping figures) with respect to their possible effect on the wool would be most interesting and instructive. However, the further discussion on this point must be allowed to stand over until we have completed our investigation on the effect of different polysulphide solutions on wool. In passing it would suffice to stress its fundamental importance, and to point out that the question of excessive dipping must be viewed not only from the point of view of an effective final concentration, but also from that of a safe final composition.

The problem of calculating the amount of polysulphide lost per animal on dipping is in its nature very similar to that of calculating the amount of thiosulphate formed per animal. Again, as each animal passes through the bath it causes a certain amount of polysulphide to be decomposed forming partially thiosulphate—chemical loss—and as it leaves the bath it carries away with in an additional amount of polysulphide—mechanical loss. In solving this problem we shall again first consider the mechanical loss.

If the initial concentration of polysulphide be P_1 gm. sulphur per 100 c.c. in an initial bath volume of V_1 deciliters, and if the final concentration be Pn in a final bath volume of Vn deciliters, the mean concentration of polysulphide sulphur in the dip wash mechanically removed from the bath will be :—

$$P_1 = \frac{(P_1 + P_n)}{2} \text{ gm. sulphur per 100 c.c.}$$

The weight in gm. mechanically removed by n animals will therefore be :—

$$\pi = \frac{(P_1 + P_n)}{2} \times (V_1 - V_n) \text{ gm. sulphur.}$$

And measuring the volumes in gallons we have :—

$$\pi = 22 \cdot 7 \ (P_1 + P_n) \ (V_1 - V_n) \text{ gm. sulphur per n animals.}$$

If now, the initial weight of polysulphide sulphur be Q_1 gm. and the final weight be Q_n gm., then the weight of polysulphide sulphur lost by chemical means will be :—

$$Q_c = Q_1 - (Q_n + \pi) \text{ gm. sulphur for n animals}$$

Hence,

$$\delta Q_c = \frac{[Q_1 - (Q_n + \pi)]}{n} \text{ gm. sulphur per animal.}$$

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Calculated on this basis, and considering in the first instance only those dips where the final dip volume after dipping was definitely known, the chemical loss per animal under different conditions of dipping has been tabulated in the following table—Table XIII.

TABLE XIII.

Effect of Dipping on Polysulphide Content.

Dip.	Initial P.S. conc. (gm. S/ 100 c.c.).	Final P.S. conc. (gm. S/ 100 c.c.).	Initial volume (gals.).	Final volume (gals.).	Initial P.S. in bath (gm. S).	Final P.S. in bath (gm. S).	P.S. lost mechan- ically. (gm. S).	Number animals dipped.	P.S. lost per animal chemically (gm. S).
TKi. 8..	1·10	0·95	390	225	19,480	9,705	7,679	536	3·9
K. 7....	0·99	0·76	700	275	31,470	9,488	16,890	805	6·3
K. 10...	1·24	0·68	800	350	45,040	10,810	19,600	900	16·3
TKi. 7..	1·41	0·65	520	325	33,290	9,592	9,118	737	19·8

From the few results recorded here the amount of polysulphide decomposed per animal dipped varies from 3·9 to 19·8 gm. sulphur. In order to study this decomposition, also in the case of those dips where the final volume of the bath was not directly given, this final volume was again calculated on the basis of an average and minimum mechanical loss per animal of 0·59 and 0·23 gallons respectively. This minimum loss basis again gives maximum figures for the chemical loss of polysulphide per animal. The results so obtained have been tabulated in Table XIV.

TABLE XIV.

Dip.	Initial P.S. conc. (gm. S/ 100 c.c.).	Final P.S. conc. (gm. S/ 100 c.c.).	Initial volume (gals.).	Number animals dipped.	P.S. de- composed per animal (0·59 basis) (gm. S).	P.S. de- composed per animal (0·23 basis) (gm. S).
K. 9.......	1·16	0·32	600	2,181	—	6·1
TKi. 5.....	0·89	0·44	400	775	—	8·2
N. 3.......	0·82	0·77	625	160	8·2	8·6
N. 12.....	0·84	0·53	500	549	8·6	11·2
N. 9.......	0·65	0·30	390	458	9·5	11·7
K. 6.......	1·43	0·81	800	1,444	—	12·4
Tvl. 7.....	1·44	0·85	950	1,487	10·4	14·1
K. 3.......	0·97	0·25	1,700	2,739	12·1	16·5
K. 5.......	1·27	0·55	600	723	17·4	23·4
K. 2.......	0·99	0·38	600	508	24·5	29·5
F. 2.......	1·76	1·16	800	600	28·1	33·2

Using the value of 0·23 gallons for the loss of dip per animal, the maximum figures for the amounts of polysulphide decomposed per animal under different sets of dipping conditions vary between 6·1 and 33·2 gm. polysulphide sulphur.

As the polysulphide thus decomposed in the process of dipping is partially oxidized to thiosulphate and partially precipitated as elementary sulphur, the data thus far obtained may be grouped together to show how the sulphur of the decomposed polysulphide is distributed between thiosulphate and elementary sulphur under different dipping conditions.

TABLE XV.

Distribution of Decomposed Polysulphide Sulphur.

Dip.	Number animals dipped.	P.S. decomposed per animal (gm. S).	Thios. formed per animal (gm. S).	Free S. precipitated per animal. (gm. S).	Per cent. P.S. converted into thiosulphate.
K. 10............	900	16·3	9·5	6·8	58·3
K. 7............	1,444	12·4	4·9	7·5	39·9
N. 3............	160	8·6	2·9	5·7	33·7
Tvl. 7............	1,487	14·1	4·3	9·8	30·5
F. 2............	600	33·2	9·9	23·3	29·8
K. 3............	2,739	16·5	4·2	12·3	25·4
K. 5............	723	23·4	5·8	17·6	24·8
N. 9............	458	11·7	2·2	9·5	18·9
TKi. 5............	775	8·2	1·5	6·7	18·3
K. 9............	2,181	6·1	1·1	5·0	18·0
N. 12............	549	11·2	1·8	9·4	16·1
K. 2............	508	29·5	2·6	26·9	8·9
Average	12,524	14·6	4·0	10·6	27·3
Limits...	—	6·1–33·2	1·1–9·9	5·0–26·9	8·9–58 3

Table XV shows that the average amount of polysulphide decomposed per animal, calculated on a total number of animals of over 12,000, amounts to 14·6 gm. sulphur, varying between 6·1 and 33·2 gm. sulphur per animal. Of this decomposed polysulphide sulphur 1·1 to 9·9 gm., or an average of 4·0 gm., is converted into thiosulphate, forming on the average 27·3 per cent. of the polysulphide decomposed. The remaining sulphur, varying from 5·0 to 26·9 gm. and averaging 10·6 gm. per animal, is chiefly precipitated as free elementary sulphur.

Apart from the general decrease in the concentration of total polysulphide sulphur in solution, the process of dipping also causes a change in the nature of the polysulphides, as we have already shown. To some extent this change is affected by certain suint constituents reacting with the polysulphides of calcium in solution. With a view to tracing the effect of the partial base exchange due to reaction with the suint, and also with the object of studying the change in the nature of the polysulphides more closely, the effect of dipping on the monosulphide equivalent, the free polysulphide sulphur, and the calcium in solution was also studied. In Table XVI the analyses of several dips for these constituents have been tabulated.

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TABLE XVI.

Dip.	M.S.S.E. initial (gm. S/ 100 c.c.).	F.P.S.S. initial (gm. S/ 100 c.c.).	Ca. initial (gm. Ca/ 100 c.c.).	M.S.S.E. final (gm. S/ 100 c.c.).	F.P.S.S. final (gm. S/ 100 c.c.).	Ca. final (gm. Ca/ 100 c.c.).
N. 12	0·131	0·711	0·280	0·126	0·403	0·250
N. 9	0·144	0·503	0·301	0·158	0·147	0·270
N. 3	0·175	0·647	0·318	0·175	0·593	0·310
TKi. 5	0·200	0·693	0·283	0·299	0·136	0·260
K. 3	0·203	0·768	0·460	0·149	0·104	0·316
K. 2	0·215	0·777	0·329	0·174	0·208	0·278
K. 9	0·246	0·912	0·496	0·165	0·155	0·320
K. 10	0·294	0·942	0·683	0·262	0·416	0·613
K. 5	0·294	0·979	0·705	0·175	0·372	0·280
Tvl. 7	0·308	1·134	0·595	0·210	0·638	0·506
K. 6	0·379	1·056	0·717	0·274	0·544	0·521
F. 2	0·418	1·346	0·792	0·308	0·756	0·555

M.S.S.E.   Monosulphide sulphur equivalent.
F.P.S.S. .   Free polysulphide sulphur.

The table shows that in all cases the process of dipping results in a decrease in the concentration of free polysulphide sulphur and calcium. This also holds for the monosulphide sulphur equivalent, except in the case of dips TKi.5 and N.9 where a small increase in the monosulphide sulphur equivalent occurs as a result of dipping.

Applying the same method of calculation as used for the total polysulphide sulphur in Tables XIII and XIV, the loss in monosulphide equivalent, free polysulphide sulphur and calcium per animal has again been calculated, the results being expressed in Table XVII.

TABLE XVII.

Dip.	Number animals dipped.	M.S.S.E. lost per animal (gm. S).	Ca. lost per animal (gm. Ca).	F.P.S.S. lost per animal (gm. S).
TKi. 5	775	— 1·8	0·4	10·0
N. 9	458	— 0·5	1·0	12·2
N. 3	160	+ 0·0	1·4	8·6
N. 12	549	0·2	1·1	11·0
K. 9	2,181	0·6	1·3	5·5
K. 10	900	0·9	2·0	15·4
K. 3	2,739	1·2	3·3	15·3
K. 2	508	2·0	2·5	27·5
K. 6	1,444	2·1	3·9	10·3
Tvl. 7	1,487	2·3	2·1	11·8
K. 5	723	3·8	13·8	19·6
F. 2	600	6·1	13·3	27·1
Average	12,524	1·4	3·4	13·2
Limits	—	—1·8–6·1	0·4–13·8	5·5–27·5

M.S.S.E. = Monosulphide sulphur equivalent.
F.P.S.S. = Free polysulphide sulphur.

The table shows that the quantity of calcium lost per animal is persistently greater than the loss in monosulphide sulphur equivalent, except in the case of dip Tvl.7. This excess of calcium loss over loss in monosulphide equivalent also holds when considered on a basis of chemical equivalent. Since the loss of this excess calcium must be counterbalanced if further loss in monosulphide equivalent as sulphuretted hydrogen is to be avoided, we have here proof that the loss in calcium on dipping must in part be affected by a process of base exchange with the alkali in the suint, the calcium being precipitated as calcium carbonate and calcium salts of suint acids, and the sulphide sulphur becoming attached to potassium. That this process of base exchange is appreciable is shown by the fact that for an average loss of 1·4 gm. in monosulphide sulphur equivalent there is a corresponding average loss in calcium of 3·4 gm.

(d) The Effect of Immersion Time on Composition.

The table also shows that there apparently exists no parallelism between the loss in monosulphide sulphur equivalent and the loss in free polysulphide sulphur. From this we may conclude that the decomposition of polysulphide on dipping is not due to oxidation to the extent one might primarily be led to expect. This point is brought out still more clearly if we consider the amount of thiosulphate formed in comparison with the loss in polysulphide. If oxidation were par excellence the dominating factor causing loss in polysulphide sulphur, then the longer the bath remained in agitation during the dipping, the greater would be the amount of thiosulphate formed, since the effect of other factors would then be minimal. That this is not so, the data tabulated in Table XVIII will show. In this table the total time taken (in minutes) for actual dipping divided by the number of animals dipped has been given as the average time per animal. This average time is taken to serve as an index of bath agitation. It must not be taken to represent the time each animal remained in the bath, since from two to five animals were immersed simultaneously. The actual immersion time per animal was two minutes as officially laid down. All figures have been given in gm. sulphur per animal.

TABLE XVIII.
Effect of Immersion Time on Composition.

Dip.	M.S.S.E. lost.	F.P.S.S. lost.	P.S.S. lost.	Thiosulphate formed.	Average time per animal (minutes).
F. 2...............	6·1	27·1	33·2	9·9	0·40
TKi. 5...........	− 1·8	10·0	8·2	1·5	0·43
Tvl. 7...........	2·3	11·8	14·1	4·3	0·44
K. 5.............	3·8	19·6	23·4	5·8	0·54
N. 12...........	0·2	11·0	11·2	1·8	0·60
K. 10...........	0·9	15·4	16·3	9·5	0·63
N. 9.............	− 0·5	12·2	11·7	2·2	0·65
K. 3.............	1·2	15·3	16·5	4·2	0·67
K. 6.............	2·1	10·3	12·4	4·9	0·70
K. 2.............	2·0	27·5	29·5	2·6	0·71
K. 9.............	0·6	5·5	6·1	1·1	1·07
N. 3.............	0·0	8·6	8·6	2·9	1·50
Average........	1·4	13·2	14·6	4·0	—

(e) THE EFFECT OF OXIDATION ON DECOMPOSITION.

If, according to the equation

$$2\ CaS_{4\cdot6} + 3\ O_2\ \rightarrow\ 2\ CaS_2O_3 + 2\ S_{2\cdot6}$$

the decomposition of the polysulphide as a result of dipping were due solely to atmospheric oxidation, then the percentage of decomposed polysulphide sulphur converted into thiosulphate must remain constant and equal to about 43 per cent., unless the thiosulphate formed is also partially decomposed. We have found, however, no indication that such decomposition of already formed thiosulphate actually occurs. On the other hand, this thiosulphate conversion constant is a linear function of the atomic ratio of calcium to sulphur in the initial polysulphides present.

If we start with a polysulphide CaS_5, then, according to the equation : —

$$2\ CaS_5 + 3\ O_2\ \rightarrow\ 2\ CaS_2O_3 +\ 6\ S$$

the conversion to thiosulphate will be 40 per cent. If, again we start with a polysulphide $CaS_{2\cdot5}$ then, accoring to the equation : —

$$2\ CaS_{2\cdot5} + 3\ O_2\ \rightarrow\ 2\ CaS_2O_3 + S$$
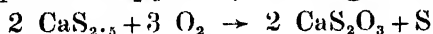
the conversion will be 80 per cent. As the atomic ratio of calcium to sulphur falls continually during the course of dipping, a comparison of the amount of polysulphide sulphur actually converted into thiosulphate, with the amount that should be converted theoretically if oxidation were the only cause of decomposition of the polysulphide, becomes possible only if we take into consideration the initial and final values of this ratio.

In the equation :

$$2\ CaSx + 3\ O_2\ \rightarrow\ 2\ CaS_2O_3 + (2x - 4)\ S$$
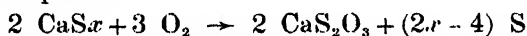
where x_1 and x_2 are the initial and final atomic ratios respectively, average or mean figures for a definite set of dipping conditions will be obtained when x is taken to be equal to $\frac{1}{2}\ (x_1 + x_2)$. We thus obtain the figures recorded in Table XIX.

TABLE XIX.

Effect of Oxidation on Decomposition.

Dip.	Initial X.	Final X.	Average X.	Theoretical per cent. conversion.	Per cent. conversion found.	Per cent. of theoretical conversion.
K. 2.......	4·61	2·20	3·40	59·0	8·9	15·1
TKi. 5.....	4·46	1·45	2·95	67·8	18·3	27·0
K. 9.......	4·71	1·94	3·32	60·2	18·0	30·0
N. 9.......	4·49	1·93	3·21	62·3	18·9	30·3
N. 12......	4·66	4·20	4·43	45·1	16·1	35·7
K. 3.......	4·78	1·70	3·24	61·7	25·4	41·2
K. 5.......	4·34	3·12	3·73	53·3	24·8	46·5
F. 2.......	4·22	3·45	3·83	52·2	29·8	57·1
Tvl. 7.....	4·68	4·04	4·36	45·8	30·5	66·6
K. 6.......	3·78	3·00	3·39	59·0	39·9	67·6
N. 3.......	4·70	4·38	4·54	44·0	33·7	76·6
K. 10.....	4·21	2·58	3·40	59·0	58·3	98·8

From the above table it will be seen that the amount of thiosulphate actually formed in the course of dipping varies from 15 per cent. to almost 100 per cent. of the amount theoretically possible if all polysulphide decomposition were due to oxidation. Thus in dip K.10 the dominant cause of decomposition of the polysulphides was almost exclusively limited to oxidation; on the other hand, in the case of dip K.2 the rôle played by oxidation is small; by far the major part of the decomposition actually found must be ascribed to other influences. As we have already pointed out, these other influences are various. To some extent the polysulphide decomposition is due to the effect of carbon dioxide and suint constituents. To what extent chemical equilibria and composition as well as concentration exert their influence cannot be determined from such a field study. It would appear, however, that to some extent the factors causing such wide fluctuations are intimately and inseparably connected with the material dipped, which again are controlled by various factors of stock-breeding and stock-raising and all they imply. As far as oxidation of the dip is concerned, it appears to us that this oxidation is primarily caused by the oxygen brought into the bath with the animal; the effect of free atmospheric oxygen due to agitation would appear to be of minor consequence. As to the form in which the oxygen, imparted to the dip by the fleece, exists, we can at present only speculate. However, it seems feasible to suppose that the fleece may contain loosely bound oxygen, attached to suint and fat constituents in a form similar to the peroxide linkage, in addition to ordinary free atmospheric oxygen. These aspects are under consideration.

IV. SUMMARY.

1. The results of analyses of lime-sulphur dipwashes, the lime and water used in their preparation, received from various parts of the Union, have been summarized and discussed. The samples referred to were all obtained from actual dippings undertaken by officials in the combation of sheep scab.
2. The preparation of lime-sulphur solutions and factors influencing its composition are detailed.
3. The effects of the processes involved in the actual dipping on the dipwash itself have been studied.
4. Additional evidence that considerable changes occur in the tankwash as a result of dipping have been obtained.
5. Factors producing these changes have been touched upon.

V. ACKNOWLEDGMENTS.

REFERENCES.

Only publications referred to in the text have been given:—

GREEN, H. H. (1915). The Sulphur Sheep Dips. *Dir. Vet. Res.*, *3rd and 4th Repts.*, pp. 120, 138, 184 and 194.

PATEL, SEN-GUPTA, AND CHAKRAVARTI (1930). Action of Sulfur Monochloride on Mercaptans. II, Formation of Organic Trisulfides and Hexasulfides. *Jnl. Indian Inst. Sci.*, Vol. 13A, p. 73. *Chem. Abstr.*, Vol. 24, p. 4770.

VAN ZYL, J. P. (1927). Notes on the Decomposition of Diluted Polysulphide Dips. Dir. Vet. Ed. & Res. 11th and 12th Repts., p. 139.

GRAF, H., AND WILKEN-JORDEN, T. J. *Researches into Dips and Dipping* (1932). Paper A. I, General Introduction. Lime-Sulphur Dips. *Dir. Vet. Serv. and An. Ind.*, *18th Rept.*, p. 1005.

WILKEN-JORDEN, T. J. (1932). Paper A. II, Lime-Sulphur Dips. A New Laboratory Method of Chemical Analysis. *Dir. Vet. Serv. and An. Ind.*, *18th Rept.*, p. 1015.

WILKEN-JORDEN, T. J. (1932). Paper A. III, Lime-Sulphur Dips. A Preliminary Study of a Colorimetric Method as a Rapid Means of Control of Polysulphide Solutions. *Dir. Vet. Serv. and An. Ind.*, *18th Rept.*, p. 1029.

WILKEN-JORDEN, T. J. (1933). Paper A. IV, Lime-Sulhpur Dips. Further Studies on the Colorimetric Method as a Rapid Means of Control of Polysulphide Solutions. Art. 31, *This Journal*, p. 231.

BEDFORD, G. A. H., AND WILKEN-JORDEN, T. J. (1933). Paper A. V, Lime-Sulphur Dips. The Minimum Effective Concentration of Lime-Sulphur Dips for Sheep Scab Eradication. Art. 32, *This Journal*, p. 239.

Researches into Dips and Dipping.

C. Miscellaneous.

The Effect of Dosing Aloes to Tick-Infected Cattle.

By

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For a number of years it has been a common belief amongst farmers in South Africa that cattle and other domestic animals, also poultry, can be kept free from ticks by giving them a daily ration of aloes. Tests were therefore carried out in order to confirm or negative this belief, although it must be admitted that keeping animals free from ticks by making them obnoxious to these parasites would not be of any great practical assistance to farmers in controlling tick-life on their farms, except in exceptional circumstances, owing to the fact that ticks can live for long periods without food, and tick-life would continue to increase owing to the opportunities these parasites have of getting on to wild animals and birds on the veld. This, however, would not apply to permanent parasites, such as the sheep ked, which are entirely dependent upon their hosts for their existence and cannot live off them for any length of time.

The only practical method of dealing with the majority of cattle ticks in South Africa is therefore to use animals as bait for attracting the ticks, and then to kill the ticks by means of regular dipping.

Experiment S. 4948.

Test No. 1.—Five cattle were sent to a tick-infested farm where they remained until they were sufficiently badly infested. They were then brought to the Laboratory and kept in a tick-free stable. The ticks attached beneath their tails were counted daily and three of the animals were dosed with aloes, which were given in the form of aloetic balls *per os* on the day they were brought in, the remaining two animals acting as controls. The result of this test is shown in the following table:—

Cattle.	Ticks.	9/1/33.	10/1/33.	11/1/33.	12/1/33.	13/1/33.
No. 2994......... Weight: 1,045 lb.. Dose: 8 dr. aloes..	*R. evertsi*......... *H. aegyptium*......	25 } 70 45	25 } 70 43+2	23+1 } 67 40+3	18 } 55 34+3	16 } 50 31+3
No. 3684......... Weight: 770 lb.... Dose: 12 dr. aloes.	*R. evertsi*......... *R. appendiculatus*... *H. aegyptium*...... *A. hebraeum*.......	32 4 } 77 34 7	32 4 } 77 34 7	32 2 } 71 27+3 7	32 2 } 65 22+2 7	29 1 } 57 20+1 6
No. 2765......... Weight: 1,200 lb.. Dose: 15 dr. aloes.	*R. evertsi*......... *R. appendiculatus*... *H. aegyptium*...... *A. hebraeum*.......	15 14 } 71 39 3	12 10 } 61 35+1 3	12 9+1 } 59 32+3 2	11 8 } 53 32 2	11 5 } 50 30+2 2
No. 2714......... Weight: 1,200 lb.. Control.............	*R. evertsi*......... *R. appendiculatus*... *H. aegyptium*......	13 2 } 36 21	10 2 } 32 20	10 2 } 29 17	9 1 } 26 16	9 1 } 24 14
No. 2805......... Weight: 1,195 lb.. Control.............	*R. evertsi*......... *R. appendiculatus*... *H. aegyptium*...... *A. hebraeum*.......	11 3 } 38 18 6	11 3 } 38 17+1 6	6 2 } 30 15+1 6	6 1 } 26 13 6	6 1 } 24 11 6

23+1 means that there were 24 ticks present, including one engorged female which was removed.

The daily decrease in the number of ticks was due, if not entirely, in a very large measure to the fact that engorged females were continually dropping off.

Result: This test demonstrated that single doses of from 8 to 15 dr. aloes to cattle do not cause the ticks to leave their hosts, and no dead ticks were found on the animals.

Test No. 2.—After the animals had been sent back to the farm for re-infection a second test was commenced on January 23rd, 1933, which differed from the first test in that the cattle were dosed daily with Cape aloes enclosed in gelatine capsules for five days, No. 2994 receiving 30 gm. aloes, No. 3684 received 45 gm. aloes and No. 2765 received 60 gm. aloes daily from January 23rd to the 27th. Furthermore, the ticks were not counted, but a rough estimate was made daily of the numbers present on various parts of the bodies. The same two control animals as were used in the first test were also used for this test. It may also be noted that this regular dosing of the animals produced marked purgation.

Result: Up to two weeks after the test was commenced the ticks appeared to be as numerous on the dosed animals as on the control animals, and when the test was concluded they appeared to be almost as numerous as they were at the commencement of the test, although all the females, which were considerably less numerous than the males, had fed and dropped off.

SUMMARY AND CONCLUSIONS.

The tests demonstrated that single doses of from 8 to 15 dr. aloes and daily doses for five days of from 30 to 60 gm. aloes to cattle do not cause ticks to leave their hosts, and no dead ticks were found on the animals.

Apart from the negative effect on the ticks, the disadvantageous effect on the animals in producing marked purgation makes this treatment most undesirable.

Researches into Dips and Dipping.

D.   Effects of Dips on Wool.

Paper I. The Effect of Arsenical Dips on Wool.*

By

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CONTENTS.

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I. INTRODUCTION.

The problems relating to the various processes in the manufacturing of woollen materials have during recent years received a great deal of consideration, particularly those processes which involve the use of chemical substances injurious to the wool fibre, and which may modify certain natural properties of wool, thus interfering with the ultimate usefulness of the finished fabric. It is a well-known fact that the main qualities of wool only become fully revealed at the spinning and dying stages, while from the wool-grower's point of view the relative resistance to the conditions leading up to those stages is an important factor. Again, the amenability of different wools to such processes must reflect back on their production. The aim of the producer should, therefore, be focussed, not only towards the production of a wool fibre of desired length and general quality, but also towards the preservation of those natural properties which determine its value as a textile material.

Of the various factors which influence or modify the physical and chemical properties of the wool fibre, the wool producer is in the first instance concerned with the effect of various chemical preparations in the form of dipping fluids. It is no exaggeration to state that the very existence of a wool-bearing sheep industry in South Africa is dependent on the effective combating of various external parasites. It was soon realized that the deleterious effect of uncontrolled parasitic infection was a far more serious factor in wool production than the damage which might conceivably be wrought by the chemicals used as dips. To illustrate this, we have reproduced two photographs, one of a sheep badly infected with scab (-*Psoroptes communis*) (Fig. 1), and the other (Fig. 2) of a staple of wool showing the effect of scab as also the healthy new growth after the animal had been cured by dipping in a lime-sulphur solution.

Fig. 1.
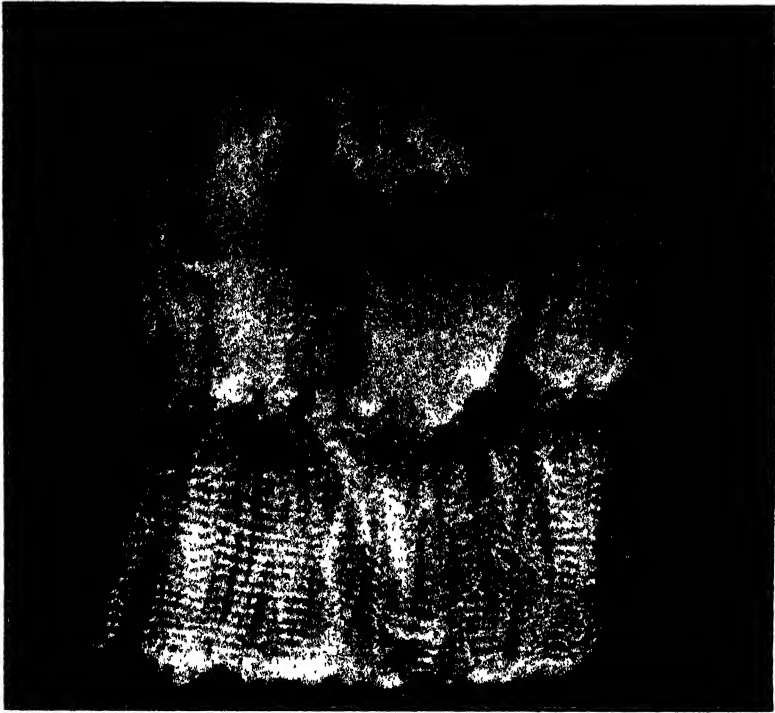
Fig. 2.

These photographs speak for themselves. Hence it is only natural to find that all initial efforts were mainly directed towards the finding of efficacious dips, and that the possible effect of such dips on the fibre was allowed to recede into the back-ground.

The selection of the chemicals used as dips is in the first instance controlled by the nature of the parasite to be exterminated. Thus lime-sulphur is highly efficacious against scab, but quite ineffective against ticks. On the other hand, sodium arsenite is sure to kill the tick, but is not of much use in combating scab. In the present paper we propose to deal only with those arsenical dips most widely used for sheep dipping, namely, arsenite of soda, used for tick destruction (Heartwater), and Cooper's Powder dip used primarily against scab. However, in reviewing the work done on the effect of these dips on the fleece in general and on the wool fibre in particular, it would appear desirable to include also other dips for the sake of comparison.

In 1913 tests were carried out by the Union Department of Agriculture to determine the possible effect of such dips as Cooper's Dip, Lime and Sulphur dips, Little's dip, tobacco extract, etc., on the scouring, combing, spinning, dying and finishing properties of the dipped wools. Samples of the dipped and undipped wools were submitted to the Bradford Technical College and Leeds University to be put through the different processes of manufacture. The

completion of the study of the samples submitted was unfortunately delayed for many years, and it was not until 1926 that a report by Hollis was submitted to the Department. The conclusions arrived at by Hollis as a result of this study may conveniently be summarized as follows:—

(1) Dips have some detectable injurious action on wool.

(2) Staining action by the dip does occur, and in some cases the discoloration is permanent.

(3) Dipped wools scour less easily than undipped wools.

(4) To some extent dipping seemed to affect the affinity of wool for different dyes, though the results were often contradictory.

(5) Defects in the fibre due to irregular application and penetration of the dipping fluid could not be definitely shown, although it was suggested that this irregularity was probably the cause of irregular dying of the finished material.

In 1915, Green, basing his study on macroscopic and microscopic examinations of the pure wool fibre, made some observations on the effect of lime-sulphur dips on wool. Wools subjected to more severe tests in which clean wool samples were steeped into solutions of lime-sulphur of varying concentrations (1-8 per cent. polysulphide sulphur), showed rapid swelling and pronounced structural changes with the higher concentrations and long exposure; while the usual concentrations and the short immersion period of two minutes showed no detectable effect on the wool, no difference being noticeable between dipped and undipped samples.

In 1931 further sheep and wool dipping experiments were carried out at the Bathurst Experimental Farm, these experiments extending over a period of 12 months. During this time groups of 30 sheep were dipped regularly every week, one group being dipped in a 0·16 per cent. solution of sodium arsenite, another group in pure water, and the third group remaining undipped for the purpose of control. The object of this experiment was to determine (1) whether Merino sheep could withstand and adapt themselves to such regular dipping, and (2) the effect of such dipping on the fleece. It was found that the process of regular dipping had no effect on the condition of the sheep, but produced marked changes in the wool. The staples from the dipped sheep were for the most part harsh and inelastic to the feel. The foreign matter consisting of sand and dirt also extended more or less throughout the length of the staple instead of being restricted to the tip, producing a general discoloration and dullness. This effect appeared to be as marked with the water dipped sheep as with the arsenite dipped animals. On the other hand, dipping appeared to have no effect on fibre fineness and contour, although the arsenite of soda did appear to lower the breaking strength of the fibre. Samples of these wools were forwarded to us for the chemical study of damage, but unfortunately the samples were found to be useless since not sufficient care had been exercised in classification and sampling.

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From this brief review it will be seen that very little attention has been paid to the possible effect of dipping on the wool fibre. Regarding the chemical effects of dip constituents on the fibre we know practically nothing. It is true that at one time certain buyers and manufacturers in the textile trade raised a great outcry against the use of certain dips in South Africa, but this outburst can only be ignored since it was based on misunderstanding and ignorance regarding the most elementary chemical principles. In the present paper we have concerned ourselves only with the chemical examination of dipped fibres. Physical measurements such as elasticity, breaking strength, etc., have not been undertaken, since other arrangements have been made for such physical studies.

II. EXPERIMENTAL TECHNIQUE.

Several aspects of the effect of dips on the wool fibre have been studied. These include the effect of dipping on hygroscopicity, the adherence of dip constituents to the wool fibre, the effect of dipping on the yield of clean wool, and the effect of such dips on the soundness of the wool fibre.

(a) MOISTURE DETERMINATION.

The analytical sample was obtained by taking small sub-samples from the original sample. One or two grams of the sample thus obtained were weighed out into a small weighing bottle with ground glass stopper. Three to four of these weighing bottles were then slipped into the vacuum tube of the apparatus reproduced below (Fig. 3).
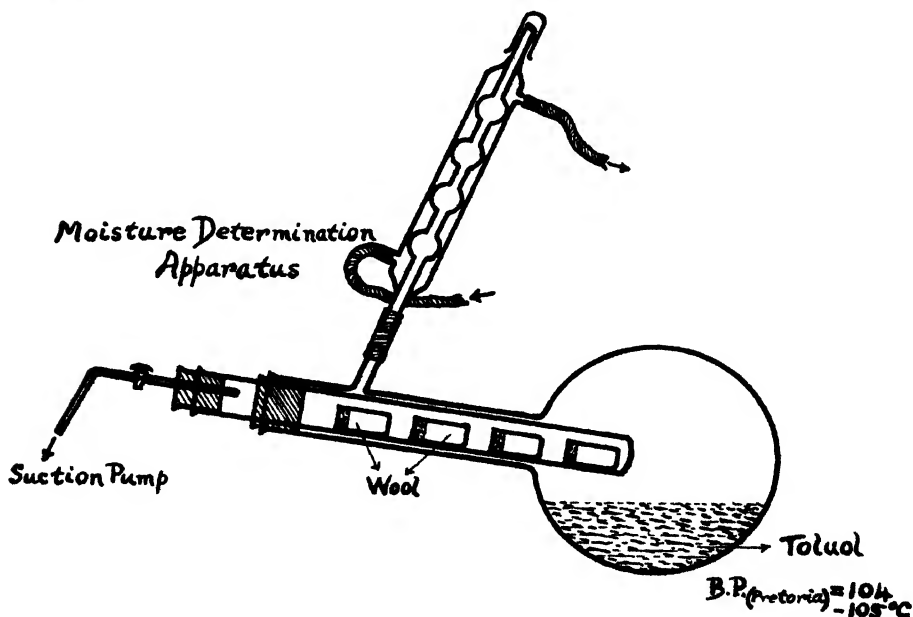
Fig. 3.

The moisture was expelled under vacuum by heating to 104-105° C. by means of toluol vapour. Constant weights were usually obtained after about 16 hours heating. The weighing bottles were then closed, allowed to cool in a desiccator, and weighed, the moisture being calculated from the loss in weight.

(b) Estimation of Arsenic in Wool.

The same method of sampling was used for determining the arsenic in the wool. The method used was that described by Green (1918) for the determination of arsenic in organic materials. Five gram samples were weighed out into a silica dish, and thoroughly wetted with ca. 30 c.c. of a 20 per cent. magnesium nitrate. The dish was then kept on a sand-bath until complete dryness and then ignited in an electric oven at a temperature just approaching a dull red heat. The ash was dissolved in ca. 20 c.c. of a 1 in 3 sulphuric acid, transferred to a 100 c.c. measuring flask, and made up to volume. Of this solution 10 to 50 c.c. aliquots were used for the actual arsenic determinations. The arsenic was evolved as AsH_3, and absorbed in a ca. $N/50$ solution of silver nitrate. In this solution the arsenious acid formed was then titrated with $N/495$ iodine, after disposing of the silver nitrate by adding sodium bicarbonate and excess potassium iodide.

1 c.c. $N/495$ J thus gives 0·1 mgm. As_2O_3.

(c) Estimation of Wool Grease, Suint and Dirt.

The method using a soxhlet extractor as described by Bonsma (1930) was tried out. It was found, however, that this method was inapplicable especially to sub-samples taken from dipped wools, since the dirt became distributed throughout the entire wool mass. Even removal of the sample from the soxhlet and washing in a basin with water failed to give a perfectly clean wool. It was, therefore, decided to go back to the older and perhaps more crude method of washing the wool in open basins. The results were certainly more satisfactory for our purpose. A 25 gm. sample was dried for 24 hours in a steam oven, and then placed in a large porcelain dish and covered with benzol which had been redistilled. The wool was carefully triturated with a forceps, thoroughly squeezed out, and the process repeated in a second dish with fresh benzol. Usually this washing process was repeated a third time. The washings were then collected in a distillation flask and the large excess benzol removed by distillation, thus concentrating the grease extract down to about 100 c.c. This concentrated solution was then filtered through a weighed quantitative filter into a 250 c.c. measuring flask, and the sludge remaining on the filter well washed with benzol. The filtrate was then made up to volume with benzol. In this solution the grease was determined by pipetting out 100 c.c. into a weighed evaporating dish, driving off the benzol on the steam bath, and weighing the residue. In another aliquot the arsenic was determined by evaporating off the benzol, evaporating down with magnesium nitrate, and igniting. For the rest the same procedure was followed as described under the determination of arsenic in wool. In the case of wool dipped in Cooper's dip the free sulphur extracted with the grease was further determined in a third aliquot by using both the Rimington-Benedict-Denis (1930) and peroxide fusion methods.

Having removed the grease, the dried wool was next washed several times with distilled water as above, the washings collected, evaporated down to about 100 c.c. and again made up to a definite volume after filtering through a second weighed quantitative filter. In an aliquot of this solution the suint was determined by evaporating down on a steam oven to constant weight. In a second aliquot the arsenic was determined by the same method as described for the wool.

For determining earth, sand and other impurities the two weighed filters containing these substances, as well as any remaining plant material subsequently removed by hand, were transferred to a weighed platinum dish, and heated in a steam bath to constant weight. The arsenic in this residue was determined as already described by evaporating down with a solution of magnesium nitrate and igniting.

The clean wool obtained by this process is allowed to dry in the atmosphere, and then weighed. At the same time its moisture content is determined on a small sample. All figures may thus be calculated, either on an absolutely dry basis, or on a basis of the same moisture content throughout.

(d) Estimation of Chlorine.

The chlorine in the raw wool was determined by the usual microtitration method in use in this Institute (Malan and v. d. Lingen, 1931) by evaporating down 2 gm. wool in a platinum dish with 10 c.c. of a 10 per cent. calcium acetate (chlorine-free) solution and igniting at a low temperature. The extract of the ash in a 5 per cent. nitric acid solution was filtered through a small filter and made up to a convenient volume. Of this an aliquot was titrated by adding excess $N/25$ silver nitrate, shaking briskly to coagulate the silver chloride formed, and then titrating back with $N/50$ ammonium sulphocyanide, with a solution of ferric alum as indicator.

(e) Chemical Estimation of Damage.

In considering the methods available for determining quantitatively the degree of damage of wool fibres, several methods were studied, including that of Sauer (1916) and the two D.I. methods described by Krais and Schleber (1929). Of these the Pauly-Rimington diazometric method and the borax method as described by Krais and Schleber (1929) were found to give the most reliable results.

The method of Pauly (1904) is based on the coupling of certain amino-acid residues with p-phenyl diazonium sulphonate thereby producing an intense reddish-brown coloration. This reaction has been made quantitative by Rimington (1930) by dissolving the diazo colouring formed in alkali, and matching the solution thus obtained against a standard solution of " New Acid Brown S ".* In this way the number of units of damage could be determined for each wool.

In the borax method as modified for our purpose, 25 c.c. of a boiling 2·5 per cent. borax solution was allowed to act for half an hour on 100 mgm. wool, and the amount of nitrogenous material thus extracted calculated as a percentage of the total nitrogen present. The extract was filtered, made up to a known volume, and the nitrogen in a suitable aliquot determined by the micro-kjeldahlization method of Folin (1919). The ammonia so formed was micro-distilled over with caustic soda by the method used by Folin and Svedberg (1930) for urea determinations, and determined by nesslerization in the usual way. The total nitrogen in the wool was determined by the ordinary macro-Kjeldahl method, using copper sulphate as catalyst.

In the Sauer method described by Krais and Schleber (1929) the amount of extractable nitrogen is determined by allowing a mixture of N/2 potassium hydroxide and a 1 per cent. hydrogen peroxide to act on the wool for 3 days at room temperature. In our micro-modification of the method 100 mgm. wool was allowed to soak for a period of three days in a solution consisting of 8 c.c. water, 10 c.c. of a 1 per cent hydrogen peroxide, and 2 c.c. of a N/2 potassium hydroxide contained in small Erlenmeyer flasks, the flasks being closed with thistle funnels containing a very dilute solution of sulphuric acid. The extract was again filtered, the solution in the thistle funnel added, made up to a definite volume, and the nitrogen in an aliquot determined by micro-kjeldahlization, distilling over with caustic soda, and nesslerization. The " soluble nitrogen " was again calculated as a percentage of the total nitrogen.

III. MATERIAL USED IN INVESTIGATION.

Two sets of materials were used in the present investigation— viz. wool samples obtained from actual dipping trials, and wool samples from undipped sheep for in-vitro studies.

The first set of samples was obtained from dipping experiments carried out at the Grootfontein School of Agriculture. Seven groups, each consisting of 50 unshorn Merino wethers, uniform in age and wool, were subjected to the following treatment:—

Lot CC3—dipped 3 times in Coopers.

Lot C3—dipped 3 times in Coopers.

Lot C2—dipped 2 times in Coopers.

Lot As3—dipped 3 times in Arsenite of Soda.

Lot As2—dipped 2 times in Arsenite of Soda.

Lot C (100 animals) used as control and not dipped.

No definite order was observed in dipping the various groups, except that Lot CC3 were always put through the dip prior to Lots C2 and C3. The dips were prepared freshly for each dipping, the interval between the first and second dipping being 10 days, and that between the second and third 14 days.

With both the sodium arsenite and the Cooper's dip the strength of the bath was regulated to show 0·16 per cent. As_2O_3, throughout the experiment, six animals being lowered into the bath at a time

and kept immersed for 1½ minutes. The weather conditions remained good, bright sunshine prevailing throughout the course of the experiment. Two days after the third and last dipping, however, heavy rains were experienced amounting to 2·8 inches. Just prior to the first dipping a shoulder sample was taken from each sheep. A similar set of samples was taken three months after the first dipping, or more than two months after the last dipping. Thereupon the sheep were shorn, the wool yields noted, and each fleece examined in the usual commercial way, observations being made as to soundness, colour, quality and feel. The live weights of the animals were also controlled by weighing the animals just prior to dipping, 10 days after the last dipping, and again just prior to shearing. The shoulder samples taken were forwarded to us for chemical examination. Unfortunately the individual sheep were not numbered, so that it was impossible for us to determine the relation of the dipped wool to the undipped sample from the same animal.

This omittance on the part of those responsible for the experiment at Grootfontein made an accurate determination of the effect of dipping on the wool most difficult. The results of this part of our investigation must, therefore, be regarded merely as preliminary and indicative. For more accurate data these and other dipping experiments will be repeated at Onderstepoort in the near future.

The second set of samples, which were used for in-vitro studies in the laboratory, were obtained from sheep at Onderstepoort which had not been dipped, at least not during the period of growth of the fleece in question. These samples were selected most carefully, in a manner to be described more fully later.

IV. INVESTIGATION OF GROOTFONTEIN SAMPLES.

Before proceeding to deal with the results of our chemical investigation of the samples submitted, it is perhaps advisable to state briefly what observations were made at Grootfontein.* In Table I the average live weights of the different groups throughout the course of the experiment have been recorded.

TABLE I.

Live Weights in Pounds.

Date.	CC3.	C3.	As3.	C2.	As2.	Control.
4.12.31	57·4	59·0	59·7	59·2	59·2	58·7
8.1.32	56·9	58·2	59·6	60·7	60·9	59·8
14.3.32	65·3	65·9	67·4	68·6	68·3	66·7

It will thus be seen that the process of dipping had no noticeable effect on the animals themselves. In Table II have been recorded certain observations made on the fleece at shearing.

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* The dipping experiments as well as the examination of the fleeces at Grootfontein were made by Mr. G. S. Maré, Sheep and Wool Research Officer, Grootfontein.

TABLE II.

Shearing Results.

Lot.	Average yield per animal (grease wool in ℔.).	Soundness.	Colour.
CC3................	7·34	Some fleeces showed break	Least attractive.
C3................	7·50	Sound	Fairly attrative.
As3................	7·41	,,	Very attractive.
C2................	7·44	,,	Fairly attractive.
As2................	7·53	,,	Very attractive.
Control............	7·95	,,	Most attractive.

The yield in raw wool from the control group thus exceeds the yields from all the other groups, the difference, however, being very small. In fact, the loss of suint due to dipping may, under circumstances, account for a more marked loss in raw yield than that expressed in the above table. The more unattractive appearance of the fleeces from lot CC3 may be ascribed to the deposit of sulphur and arsenic sulphide especially noticeable in a freshly prepared bath of Cooper's dip, the animals emerging from it being quite yellow. As dipping proceeds this staining effect gradually diminishes, probably due to the chemical action of the suint in solution on the arsenic sulphide forming soluble thio-arsenites. It will be remembered that lot CC3 were always put through the freshly prepared wash prior to lot C3. Whether the breaks in the staples noted in some fleeces from lot CC3 can be ascribed to the effect of the yellow deposit on the fleece cannot be answered at this stage, as obviously further study is necessary.

Having thus briefly referred to the observations made at Groot-fontein, we may now proceed to describe more fully our chemical study of the samples.

(a) EFFECT OF DIPPING ON HYGROSCOPICITY OF WOOL.

Duplicate moisture determinations were made on samples of raw wool from both dipped and undipped fleeces by the method already described. After complete " dryness " the wool samples in the weighing bottles were placed open in a desiccator containing water, and left to condition in this saturated atmosphere for two days. The fluctuations in room temperature were small. These results have been tabulated in Table III, columns 2 and 4 representing the percentage regain at normal atmospheric temperature and humidity, and columns 3 and 5 representing the percentage of reabsorbed moisture at 100 per cent. humidity and atmospheric temperature.

TABLE III.

Effect on Hygroscopicity—Raw Wool.

Group.	Before dipping.		After dipping.	
	Percentage regain.	Percentage water reabsorbed at 100 per cent. humidity.	Percentage regain.	Percentage water reabsorbed at 100 per cent. humidity.
CC3................	6·63	30·30	6·21	21·68
C3.................	6·89	27·30	5·60	18·75
As3................	6·55	26·30	6·44	19·10
C2.................	6·64	28·50	6·99	18·85
As2................	6·34	28·65	5·95	20·08
Control............	6·50	30·63	6·36	29·38

Except in the case of lot C3 there is no appreciable difference between dipped and undipped samples at atmospheric humidity. However, at 100 per cent. humidity raw wools show a decided change in their absorptive capacity for moisture on being dipped. The absorption capacity for undipped wools is here more than 30 per cent. higher than that for dipped wools. The main reason for this change may be ascribed to the fact that some of the more hygroscopic constituents of the suint are removed from the wool in the process of dipping. As will be shown later, most of the more readily removable suint constituents are removed by the second dipping, there apparently being no appreciable difference in the suint content of twice and thrice dipped wools. In parallel with this, there is no appreciable difference in hygroscopicity between twice and thrice dipped raw wools.

Apart from the effect of suint and other constituents of raw wool, there is also the possibility that the wool fibre itself is so affected by the dip that its hygroscopicity may be changed. According to Tänzer (1930) the treatment of wool with alkalis and acids results in a loss of weight, together with a loss in affinity for moisture. Speakman (1931) showed that the treatment of wool with sodium sulphide does not result in any noticeable change in hygroscopicity for humidities below 100 per cent.; at 100 per cent. humidity, however, a small change in moisture affinity was noted, the untreated wool absorbing less moisture than the treated wool.

In order to determine whether any such change in the absorptive capacity of the wools had occurred as a result of dipping, the wool samples under investigation were carefully degreased and desuinted by washing in benzol and distilled water respectively. The moisture content of the atmosphere conditioned samples were again determined, and the dry-wool again allowed to reabsorb moisture in a desiccator at 100 per cent. humidity. The results so obtained have been tabulated in Table IV.

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TABLE IV.

Effect on Hygroscopicality—Clean Wool.

Group.	Before dipping.		After dipping.	
	Percentage regain.	Percentage water reabsorbed at 100 per cent. humidity.	Percentage regain.	Percentage water reabsorbed at 100 per cent. humidity.
CC3................	10·19	27·0	9·63	26·3
C3.................	8·87	27·0	9·26	26·5
As3................	9·55	27·4	9·42	25·5
C2.................	9·60	27·2	9·79	26·8
As2................	9·57	27·5	9·37	27·0
Control............	10·08	27·2	10·06	27·5

The relatively small changes in hygroscopicity shown by the variously treated wools measured at atmospheric humidity we do not propose to discuss here, since such changes can be stressed only when working under carefully regulated conditions of humidity in a specially equipped laboratory. The results at 100 per cent. humidity, however, show that the effects of dipping on the wool fibre were so small or of such a nature that they are not reflected by its affinity for moisture.

(*b*) ADHERANCE OF DIP CONSTITUENTS TO WOOL.

Arsenic determinations were made on the raw wool, on the suint, grease, sand and other impurities, and on the clean fibres by the methods already described. In Table V these determinations have been recorded, all figures being expressed as mgm. As_2O_3 per 100 gm. of the original raw wool.

TABLE V.

Arsenic Adhering to Wool.

Wool constituent.	CC3.	C3.	As3.	C2.	As2.	Control.	
						Before dipping.	At shearing time.
Suint............	109·0	97·0	53·0	52·5	45·0	0·0	5·0
Grease...........	30·0	27·0	15·0	24·8	10·0	0·0	0·0
Impurities.......	21·8	18·3	29·0	6·5	6·2	0·0	0·0
Clean fibre.......	21·0	14·0	16·0	16·0	19·0	0·0	0·0
TOTAL........	181·8	156·3	113·0	99·8	80·2	0·0	5·0
Raw wool.......	190·0	150·0	115·0	100·0	80·0	0·0	5·0

These results are significant, especially if it be remembered that the wool samples were taken more than two months after the last dipping. The fact that the CC3 sample shows the highest arsenic content is in agreement with the suggestion that the free arsenic sulphide suspended in a freshly prepared wash of Cooper's dip is deposited as such in the fleece. In fact, the samples dipped in Cooper's are throughout higher in arsenic than those dipped in arsenite of soda, due mainly to this depositation of the insoluble ingredients of Cooper's dip powder. The small quantity of arsenic found in the control sample taken at the conclusion of the dipping experiment, suggests that the undipped sheep of the control group had come into contact with freshly dipped sheep, thus causing slight contamination. It should further be noted that at least half of the total arsenic in the raw wool is in a fairly soluble form, being removed along with the suint. A fairly appreciable percentage, however, is or becomes very closely associated with the clean fibre, being fixed in such a way that it cannot be removed by mere washing in water. Whether this arsenic enters the fibre during the actual dipping process, or whether it enters during the period between dipping and shearing, or whether it becomes fixed in the fibre only in the actual process of suint extraction in the laboratory, it is most difficult to say.

In order to determine how this apparently fixed arsenic is affected by the process of scouring, 25 gm. samples of the raw wools were put through three successive baths of about 10 litre capacity, containing in order solutions consisting of 0·36 per cent. soap and 0·17 per cent. Na_2CO_3, 0·30 per cent. soap and 0·10 per cent. Na_2CO_3, and pure water respectively. The temperatures of the first two baths were 49° and 45° C. respectively, the third bath containing water being at ordinary temperature. The wool was washed for two minutes in each of these baths, and subsequently thoroughly rinsed with clean water. The amounts of arsenic remaining fixed in the fibre have been tabulated in Table VI.

TABLE VI.

Arsenic in Scoured Wools.

Sample.	Mgm. As_2O_3 per 100 gm. scoured wool.
CC3	14·0
C3	14·0
A3	13·0
C2	12·0
As2	11·5
Control	---

Comparing the results thus obtained with those tabulated in Table V for the clean fibre, and remembering that in these wools the clean fibre only amounts to about 50 per cent. of the raw wool, we observe that more than half of the apparently fixed arsenic is removed by the scouring process. However, speaking relatively, a still fairly appreciable quantity remains fixed even in the scoured fibre. The fate of this arsenic we hope to investigate still further,

if possible tracing it throughout the whole of the manufacturing process up to the finished and worn product. We feel it our duty, here, to sound a note of warning to buyers, manufacturers, and others concerned in the wool trade, not to resort to rash speculations regarding the interpretation of this arsenic in the textile trade. It is entirely unjustified to rush to the conclusion that this arsenic is bound to have some ill effect on the person wearing garments made from such wool. Such a conclusion can only be justified when based on the experimental facts of further research into the problem. What is more, South African wool-bearing flocks are not, as a rule, dipped in arsenical preparations, the official dip recommended against scab being a solution of calcium polysulphides containing no arsenic. The argument of ill effects to human health, if it *should* be proved by further research, would be applicable, as far as scab eradication is concerned, only to arsenic-containing preparations.

In examining the grease obtained from wools dipped in Cooper's dip, it was observed that this grease was to an appreciable extent contaminated with free elementary sulphur. On analysis the following results were obtained :—

Sample CC3 ... ... 3·10 per cent. sulphur.
Sample C3 ... ... 2·22 per cent. sulphur.
Sample C2 ... ... 1·09 per cent. sulphur.

TABLE VII.

Effect on Wool Constituents.

	Group CC3.		Group C3.		Group As3.	
	Before.	After.	Before.	After.	Before.	After.
Moisture..........	5·85	6·23	5·30	6·45	6·05	6·15
Grease...........	14·27	16·38	14·38	15·42	15·40	13·20
Suint.............	6·77	3·51	6·86	3·20	7·66	3·19
Impurities.......	25·68	23·46	24·90	23·74	22·50	25·90
Clean fibre.......	46·16	50·48	49·07	50·92	48·83	50·66
TOTAL........	98·73	100·08	100·51	99·73	100·59	99·10

	Group C2.		Group As2.		Control.	
	Before.	After.	Before.	After.	Before.	After.
Moisture..........	6·54	6·23	5·62	5·96	6·17	5·98
Grease...........	13·90	13·88	13·60	14·02	13·96	14·65
Suint.............	7·11	3·17	7·29	3·22	6·94	6·77
Impurities.......	23·88	28·32	24·53	27·60	24·15	26·72
Clean fibre.......	47·80	49·91	48·27	49·60	46·27	48·28
TOTAL........	99·23	101·51	99·31	100·83	99·47	102·40

The sulphur contents here given were calculated on a raw wool basis, so that the grease obtained from the dipped CC3 sample contained close on 20 per cent. of free sulphur. As will be shown presently this sulphur causes a quite noticeable rise in the apparent grease content of the wool as determined by the usual solvent extraction method. It is also interesting to note that normal raw wool, yielding around 50 per cent. of clean fibre, contains only from 1·5 to 2·0 per cent. sulphur consisting chiefly of cystine sulphur, while in the case of wool CC3 the free sulphur alone amounts to over 3 per cent.

(c) EFFECT OF DIPPING ON RAW WOOL CONSTITUENTS.

The grease, suint, impurities, and clean fibre contents in raw wool were determined by the methods already described. The results obtained have been tabulated in Table VII.

All the figures in the above table have been calculated on a raw wool basis. It will be seen that there is no significant difference in the analysis of the samples before dipping. The process of obtaining samples, representative of the group as a whole, by the method of sub-sampling thus far used, is therefore apparently fully justified. It would further appear that the selection of the sheep for the dipping experiments was highly efficient, since all groups show a fairly uniform fleece analysis (shoulder portion). It is also evident that there is little, if any, difference in the yield of clean fibre between the dipped and undipped wools. The small increase in the grease content of wools dipped in Cooper's dip is in all probability due to the sulphur contaminating the grease, as we have already pointed out. There is, however, a marked decrease in the suint content of all the dipped wools, this decrease roughly amounting to about 50 per cent. There is, therefore, no doubt that the process of dipping results in the washing-out of some of the suint in the raw wool. However, this leaching out process is apparently limited, since it is not appreciably greater for wools dipped three times than for wools dipped twice.

Exactly the same effect is observed when the chlorine contents of the different wools are compared. This can best be illustrated by grouping together the suint and chlorine contents with the hygroscopicity of the different wools at 100 per cent. humidity—

TABLE VIII.

Leaching Effect of Dipping.

Group.	Before dipping.			After dipping.		
	Percentage Suint.	Percentage Chlorine.	Hygroscopicity.	Percentage Suint.	Percentage Chlorine.	Hygroscopicity.
CC3........	7·19	0·22	30·30	3·74	0·14	21·68
C3.........	7·24	0·21	27·30	3·42	0·12	18·75
As3........	8·15	0·20	26·70	3·39	0·13	19·10
C2.........	7·61	0·20	28·50	3·38	0·13	18·85
As2........	7·72	0·21	28·65	3·42	0·13	20·08
Control.....	7·39	0·20	30·63	7·19	0·21	29·38

(d) Effect of Dipping on Wool Fibre.

The wool samples in question were investigated for soundness or damage by both the Rimington-Pauly diazometric method and the Sauer method. Due to the fact that the individual samples comprising the larger group samples had not been numbered, and also on account of certain important sampling considerations to be discussed later, twenty individual sub-samples, carefully degreased and desuinted, were examined from each group. The results obtained by the Rimington-Pauly method—expressed in Rimington units of damage—have been tabulated in Table IX as average figures for each set of 20 determinations.

TABLE IX.
Damage by Rimington Method.

Group.	Before dipping.	After dipping.
CC3....................................	45·0	63·2
C3.....................................	47·1	48·7
As3....................................	41·0	54·0
C2.....................................	40·2	41·2
As2....................................	45·0	40·0
Control................................	44·2	40·0

If these average figures can be relied on to give anything like an exact reflection of the true state of affairs, we must conclude that only the wools CC3 and As3 show any detectable damage. However, considering the remarkable sensitivity of the Rimington method under certain conditions, this damage would appear to be insignificant, and it is highly doubtful whether it can ever be recognized in industrial processing and manufacture. In the case of the As2 wool the fibre has rather been improved than damaged.

The results obtained by the Sauer method confirm these observations. Damage was here calculated to represent the percentage of the extractable nitrogen of the total nitrogen in the wool. The results thus obtained have been tabulated in Table X, representing average figures from 20 determinations obtained as above.

TABLE X.
Damage by Sauer Method.

Groups.	Before dipping.			After dipping.		
	Total N per cent.	Extracted N per cent.	Damage per cent.	Total N per cent.	Extracted N per cent.	Damage per cent.
CC3..............	14·0	1·75	12·5	13·9	2·00	14·2
C3...............	14·8	1·74	11·8	14·9	2·03	13·6
As3..............	14·0	1·84	12·7	14·5	2·91	20·1
C2...............	14·0	1·65	11·4	14·6	1·35	9·5
As2..............	14·1	1·85	13·4	14·1	1·90	13·4
Control..........	14·0	1·75	12·5	14·0	1·85	13·2

The fluctuations in the total nitrogen content must be ignored since the different groups were analysed at different periods under different humidity conditions. For each group, however, all the samples, including those for the determination of damage, were weighed out at the same time, so that the percentages of damage are still strictly comparable.

V. IN-VITRO STUDIES ON THE WOOL FIBRE.

With the object of obtaining a somewhat clearer insight into the various factors which might be responsible for damage to the fibre in the process of dipping in the usual arsenical dips, the matter was further followed up, testing out some of the more obvious factors under laboratory conditions of control. For this purpose a special parent sample of undipped wool was used. Even then various difficulties were at first encountered, it being impossible to obtain reproduceable results. Having satisfied ourselves that the methods of determining damage were highly reliable, our attention was next directed to the difficult question of wool sampling. It was soon found that the chemical methods used were so sensitive that they were able to reflect appreciable differences in the fibre, not only in different parts of the fleece, but also in the different staples from the same part of the fleece. In addition to this, all tips are normally far more seriously damaged than the rest of the fibre. This difficulty of sampling was eventually overcome by the following method : —

From a carefully removed shoulder portion of the fleece one gram staples were selected and the tip portions removed by cutting about half an inch from the tip, all staples being cut the same length as measured from the skin end. These staples were tied up with cotton thread, and carefully cleaned in benzol and distilled water, avoiding as far as possible all felting of the fibres. The individual analytical samples were obtained from these staples by taking small sub-samples from each staple in such a way that the thus obtained composite analytical sample amounted to about 0·10 gram. That such samples are representative and reproduceable, the following table will show.

TABLE XI.
Uniformity of Samples of Rimington Method.

Sample.	Colorimetric readings.		Rimington units of damage.
	Standard.	Wool.	
1..........................	5·0	9·5	52·6
2..........................	5·0	10·5	47·6
3..........................	5·0	10·5	47·6
4..........................	5·0	10·0	50·0
5..........................	5·0	9·0	55·5
6..........................	5·0	9·5	52·6
7..........................	5·0	10·5	47·6
8..........................	5·0	10·0	50·0
AVERAGE..............	5·0	9·81	50·4

(a) Effect of Sodium Arsenite and Cooper's Powder Dip on Wool.

A series of experiments was made by treating $0 \cdot 1$ gm. samples of the wool with 20 c.c. solutions of pure sodium arsenite (approximately Na_2HAsO_3) and Cooper's Powder Dip at the concentrations normally employed in the field. The results obtained with the usual two minute immersion revealed no measurable damage, and need not be given here. In a second series of experiments the wool samples were immersed in these solutions for two hours, and the damage studied by the Rimington method. The results have been tabulated in Table XII.

TABLE XII.

Influence of Cooper's and Sodium Arsenite.

Treatment.	Strength of solution (Percentage As_2O_3).	pH of solution.	Damage (Rimington units).
Untreated sample..........	—	—	$50 \cdot 4$
Sodium arsenite...........	$0 \cdot 08$	$9 \cdot 57$	$52 \cdot 6$
,, ,, ...	$0 \cdot 16$	$9 \cdot 90$	$52 \cdot 6$
,, ,, ...	$0 \cdot 24$	$10 \cdot 24$	$55 \cdot 5$
Cooper's powder...........	$0 \cdot 13$ (soluble)	—	$50 \cdot 0$
,, ,, ...	$0 \cdot 07$ (,,)	—	$50 \cdot 0$

It will thus be seen that no damage can be demonstrated, not even after two hours immersion. The above results, however, have been obtained with a fairly pure sample of sodium arsenite. In order to determine whether these results may in any way be influenced by the proprietary brands of sodium arsenite in use, the effect of various brands of sodium arsenite was further studied, using solutions containing $0 \cdot 16$ per cent. As_2O_3, and immersing for two hours.

TABLE XIII.

Effect of Proprietary Arsenite Brands.

Brand.	Percentage As_2O_3 in solution.	pH of solution.	Damage (Rimington units).
Kynoch.................	$0 \cdot 16$	$9 \cdot 3$	$58 \cdot 7$
Protea.................	$0 \cdot 16$	$9 \cdot 3$	$52 \cdot 6$
Conquest...............	$0 \cdot 16$	$8 \cdot 9$	$50 \cdot 0$
Steward................	$0 \cdot 16$	$8 \cdot 9$	$50 \cdot 0$

These figures show that all the proprietary brands studied are quite safe as far as damage to the wool fibre is concerned.

It might, however, be argued that under certain conditions, due to faulty preparation of the dipping bath, the concentration of the dip wash may rise above the specified strength. Accordingly this point was also investigated, the concentration of the solutions being increased from 0·08 to 1·6 per cent. As$_2$O$_3$, and the wool samples in each case being immersed for two hours.

TABLE XIV.

Influence of concentration on Damage.

Concentration (Percentage As$_2$O$_3$).	pH of solution.	Damage (Rimington units).
0·08	9·6	52·6
0·16	9·9	52·6
0·24	10·2	55·5
0·80	11·0	77·0
1·60	11·1	83·0

These results show that as a result of immersion for two hours in a solution of sodium arsenite containing 0·8 per cent. and more As$_2$O$_3$, the wool is clearly damaged. But even then it is extremely doubtful whether any measurable damage will occur when the wool is immersed for the usual two minutes. What is of definite interest, however, is the observation that this increase in damage coincides with a definite increase in pH or hydroxyl ion concentration. It is a well-known fact that caustic alkalis have a deleterious effect on the wool. It is, therefore, very important that the effect of increasing hydroxyl ion concentration on the wool fibre should be further studied. The importance of this aspect of the matter is not limited to the use of substances with alkaline reaction such as dips, but applies with equal force to certain stages in the industrial processing of the wool.

For this and other reasons a series of experiments was made using solutions of sodium arsenite of increasing hydroxyl ion concentration. The concentration of the arsenite was kept constant at 0·16 per cent. As$_2$O$_3$, but the pH of the solutions was so changed by the addition of sodium hydroxide as to vary from 9·9 to 12·4. In order to determine the possible effect of the arsenite ions on the wool, these experiments were repeated with solutions of pure sodium hydroxide of hydroxyl ion concentrations covering the same range as the arsenite solutions. The hydroxyl ion concentrations of the arsenite solutions were determined electrometrically using the modified quinhydrone electrode described by Davis (1931). The hydroxyl ion concentrations of the solutions of pure sodium hydroxide were calculated theoretically assuming solutions weaker than tenth normal to be completely dissociated. All samples were immersed for two hours at room temperature. The determination of damage was made both by the Rimington diazometric method and by the D.I. borax method. The results thus obtained have been tabulated in Tables XV and XVI.

TABLE XV.
Effect of NaOH on Wool.

Treatment.	pH.	Rimington method damage.	Borax method *damage.
1............	10·4	49·5	4·0
2............	11·9	60·6	4·3
3............	12·4	166	4·4
4............	12·6	250	5·8
5............	13·0	500	6·8
Untreated......	—	50·4	4·0

TABLE XVI.
Effect of Arsenite + NaOH on Wool.

Treatment.	pH.	Rimington method damage.	Borax method *damage.
1..••........	9·9	47·6	3·9
2............	10·3	55·5	4·3
3............	11·3	76·9	4·7
4............	12·0	166	5·2
5............	12·4	250	6·1
Untreated......	—	50·4	3·8

We note that both the Rimington and the Borax methods demonstrate a steady rise in damage with rise in pH. The tables also show that this increase in the units of normal damage becomes noticeable only after the pH rises above a certain critical limit lying in the neighbourhood of 10·3. These results are thus in agreement with the observed† relation between hydroxyl ion concentration and tensile strength.

In graphs I and II the influence of pH on the soundness of the fibre has been illustrated graphically. Both graphs, i.e., both the Rimington and the Borax method, present the same picture. We note that both methods show that in a medium of equal pH the solution of arsenite plus sodium hydroxide shows a greater damage effect than the sodium hydroxide alone. It would, therefore, appear as if the negative arsenite ions exert some effect of their own. We suggest that this behaviour of the arsenite ions is closely connected with the absorption and apparent fixing of some of the arsenic by the wool fibre. Whether we are here dealing with mere absorption, or whether the arsenic enters into chemical combination with certain free amino-acid groups of the keratin molecule it is most difficult to say.

*This nitrogen includes that extracted by the sodium hydroxide and sodium arsenite solutions.

†Compare: Bulletin of Wool Industries Research Association, Torridon, Vol. 3, No. 1, p. 23 (1932).

GRAPH I

GRAPH II
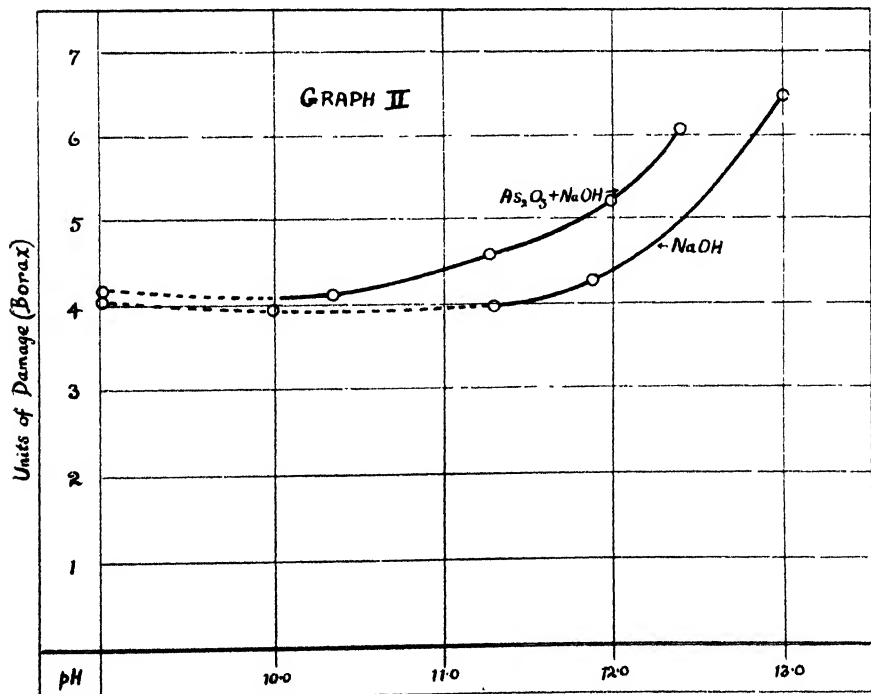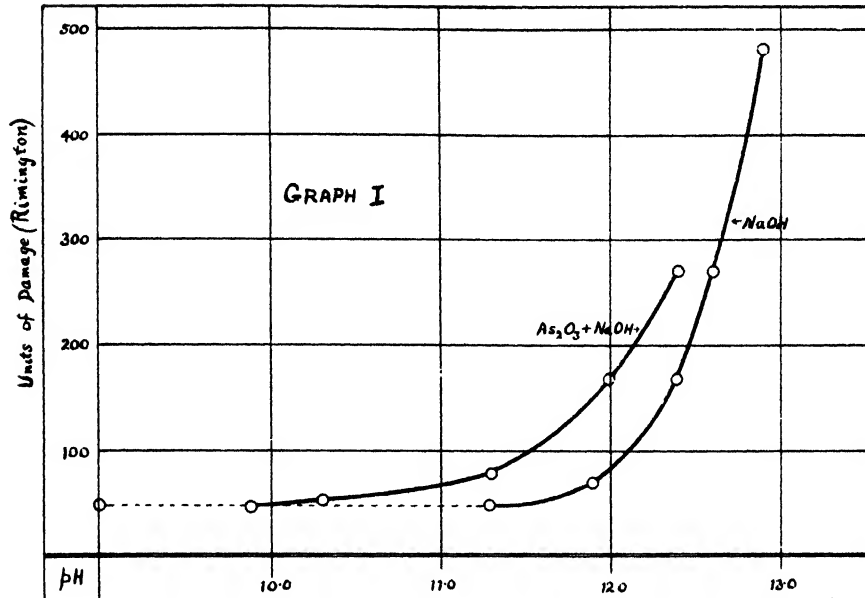
(b) The Influence of Sunlight and Ultra-Violet Radiation as a Factor in Dipping Related to Fibre Damage.

In considering the effect of dipping on the soundness of the wool, there is one very important factor which must not be lost sight of. Up to the present we have been studying the direct effect of certain dips on the wool fibre, concentrating on the direct effect of such dips as the primary cause of fibre damage. There is, however, also the possibility that the dipping effect may be only a secondary factor in wool damage. By this is meant that the process of dipping may serve to bring out certain dormant changes in the fibre which had been caused by influences other than dipping. As one of the most potent of these outside influences we venture to suggest the effect of sunlight, or more particularly the ultra-violet portion of the sun's spectrum, on the fleece. From measurements on the ultra-violet radiation intensity of South African sunlight by Osborn (1932), it has been found that the ultra-violet radiation in South Africa is decidedly high, particularly so on the high inland plateaux. Naturally such radiation would in the first instance effect the outermost exposed portion of the fleece, and it is by no means improbable that the photo-chemical action thus produced is the primary cause of the intense weathering of the tip portions of the fleece. In how far such radiation might affect the more inner portions of the fleece is most difficult to say, since we do not know to what extent the raw wool mass is opaque to ultra-violet radiation. Naturally the degree of penetration would depend on the nature and structure of the fleece.

GRAPH III

Units of Damage (y-axis)

1. Control—Untreated and Unexposed
2. Treated in Coopers— then Exposed
3. " " Arsenite— " "
4. Exposed—then treated in Coopers
5. Treated in Water—then Exposed
6. Exposed—then treated in Arsenite
7. Exposed only
8. Exposed— then in Water

Normal Damage
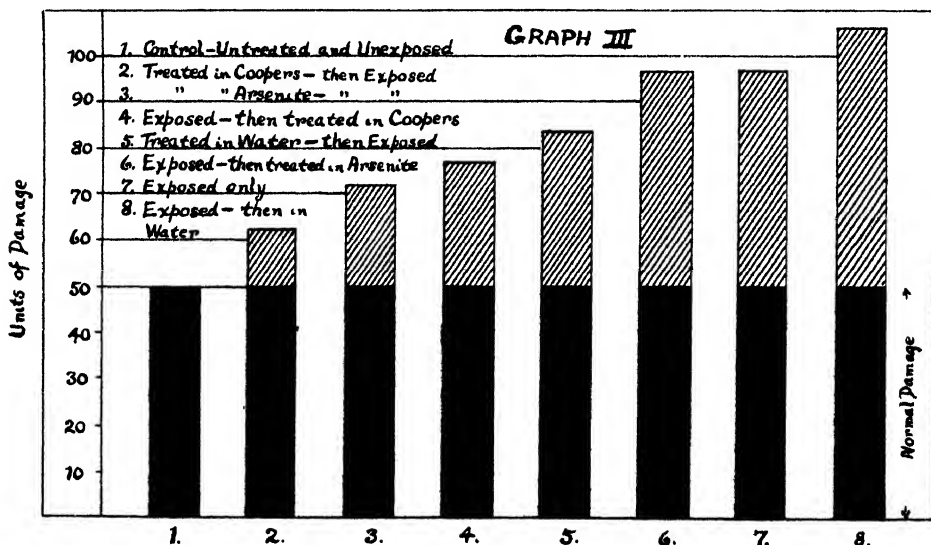
That sunlight, and more particularly the ultra-violet radiations, do affect wool and woollen goods is a daily observed occurrence and well known. What is not generally realized, however, is the close bearing this effect of sunlight may have on dipping questions in relation to fibre damage. To stress these considerations more

effectively, we have studied the effect of sunlight and ultra-violet radiation in conjunction with the arsenical dips. For this purpose two sets of experiments were made. In the first set samples of the clean fibre were exposed for an hour to the radiations of a quartz mercury-arc lamp at a distance of 18 inches, and subsequently immersed in solutions of Cooper's ($0\cdot13$ per cent. soluble As_2O_3), and sodium arsenite ($0\cdot16$ per cent. As_2O_3) as well as in pure water for two minutes.

In the second set the wool samples were first immersed in the same solutions of Cooper's, sodium arsenite, and water for two minutes, and then while still wet subjected to the ultra-violet radiation for an hour. The results obtained in duplicate have been tabulated in Table XVII and illustrated graphically in Graph III.

TABLE XVII.

Relation of Ultra-Violet to Dipping.

Experiment.	Treatment.	Damage (Rimington units).
1................	Control—not exposed and not treated........	50·4
2................	Exposed, but not treated..................	86·8
3................	Exposed and then treated with water........	105·3
4................	Exposed and then treated with Cooper's.....	76·9
5................	Exposed and then treated with arsenite.	86·8
6................	First treated with Cooper's and then exposed.	62·5
7................	First treated with arsenite and then exposed.	71·4
8................	First treated with water and then exposed...	83·3

Comparing these figures for damage with those for the same dips in Table XII, it immediately becomes evident that the ultra-violet treatment of the wool definitely affects the soundness of the fibre. Experiment 2 shows that mere exposure alone already affects the fibre, although this effect may be latent, and Experiment 8 shows that this radiation effect remains the same, whether the wool is exposed in the dry or wet state. Considering the wools first exposed and subsequently treated with the dips, we observe the most interesting fact that the treatment with pure water produces appreciably more damage than either the Cooper's dip or the sodium arsenite. Exactly the same phenomenon is observed with wools first treated and then exposed. This observation gains in practical importance when we recall that the dipping experiments carried out at Bathurst showed that the wool suffered at least as much by dipping in water as by dipping in sodium arsenite. We are not in a position at the moment to give a full explanation of this highly interesting observation. However, it seems feasible to assume that the exposure of the wool fibre to ultra-violet radiations directly affects the keratin molecule. We venture to suggest that these radiations, probably by resonation, set up abnormally violent oscillations within certain parts of the molecule, the more disturbed groups in the molecule becoming either totally detached or greatly loosened. This would explain the observation of Barritt and King (1929) that the mere exposure of wool to ultra-violet radiation causes a loss in the sulphur-content of the wool. Apart from the sulphur totally detached, a still greater

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proportion becomes loosened to such a degree that the exposed wool was found to lose about 11 per cent. of its sulphur on subsequent treatment with a very dilute alkali. We have shown the same to hold true for at least a portion of the nitrogen in the keratin molecule, as the following results will show:—

Exposure.	Percentage N. extracted (Sauer method).	Percentage of total N. in wool.
Unexposed......................	3·0	21·4
Exposed ¼ hr. at 1 ft............	4·0	29·3
Exposed ½ hr. at 1 ft...........	4·2	30·0
Exposed 1 hr. at ca. 1½ ft.......	4·6	32·9

The explanation why water affects the exposed fibre to a much greater extent than solutions of sodium arsenite and arsenate also follows readily enough from this theory. By loosening certain parts or radicals of the keratin molecule, the exposed molecules obviously exist in a state of metastability. Assuming now this destablising action of the molecule to be reversible, it is obvious that under certain conditions the metastable form may be reconverted into the stable form. Thus pure water detaches and dissolves the loosened portions of the molecule, whereas the presence of arsenite and arsenate ions in the water impedes this detaching action of the water, exerting their influence in the direction of restabilising the metastable molecules. Apparently this restabilising effect is not limited to the anions of the arsenic acids, since in the course of our experiments it was found that the anion of borax exerts a similar effect.

From Table XVII it will also be seen that the damage to the fibre is least in the case of Cooper's dip, especially where the wool was first treated and then exposed. It has already been pointed out that immersion in freshly prepared Cooper's dip results in an appreciable deposit of sulphur and arsenic sulphide on the fibres. It was therefore suspected that the yellow film thus covering the fibres might conceivably act as an ordinary filter, greatly impeding the penetration of the ultra-violet to the actual fibre. This suspicion was easily enough confirmed by treating a wool sample with a clear filtered solution of Cooper's dip, thus preventing the formation of a yellow film on immersion.

TABLE XVIII.

Influence of Staining Effect on Ultra-Violet Action.

Treatment.	Damage (Rimington units).
Not treated and exposed...........................	77·7
Treated with Cooper's and exposed..................	66·6
Treated with Cooper's clear filtrate and then exposed..	90.9

The fact that the wool sample treated with the clear filtrate of Cooper's dip showed appreciably more damage than the sample treated with the full dip in its original state, thus shows the light filtering effect of the yellow deposit formed, and explains why wools thus treated become more resistant to ultra-violet radiations. Similar observations have been made by Kertesz (1919) by protecting the fibres with a film of chromium salts.

Having thus shown that the wool fibre can be protected to some extent against the action of ultra-violet light by the application of an artificial film, it appeared highly interesting to determine to what extent the natural grease and suint on the raw wool fibre would impede the penetration of such radiations. With this object in view the influence of ultra-violet radiations on the wool in different stages of the process of cleaning was compared with that on the raw wool, the samples being exposed for one hour at a distance 18 inches from a mercury-arc lamp. After exposure the raw and desuinted wools were degreased by washing in ether, the degreased wool being desuinted with water. All the samples were finally washed in pure water. The results obtained in Rimington units of damage have been tabulated in Table XIX.

TABLE XIX.
Protective Influence of Grease, Suint, etc.

Experiment.	Wool treatment.	Damage.
1....	Raw wool—unexposed....	53
2......... ...	Raw wool—exposed.....	ca. 83
3..	Degreased wool —exposed.... .... ---.... .	ca. 83
4...... . . .	Desuinted wool—exposed.......................	ca. 83
5..	Degreased and desuinted wool—clean fibre—exposed	ca. 100

The results show that the raw and partially cleaned wools were slightly less affected by the ultra-violet treatment than the clean fibres. However, it is doubtful whether this apparent protective influence on the part of the grease and suint can be ascribed exclusively to their absorptive action of the ultra-violet. The possibility is by no means excluded that the suint *in solution* may reverse the initial photo-chemical effect already referred to. We conclude, therefore, that the protective influence of grease and suint against ultra-violet is, if anything, exceedingly small.

The exposure of wool to actual sunlight produces exactly similar effects. In the first set of experiments both clean and raw wool samples were immersed in solutions of Cooper's (0·13 per cent. soluble As_2O_3), sodium arsenite (0·16 per cent. As_2O_3), and water for two minutes, and kept out of contact with direct sunlight. After three days the wools were again immersed in Cooper's, sodium arsenite and water, and kept for another three days. In the second set of experiments the wool samples were exactly similarly treated by immersing twice at an interval of three days, with the exception that these samples were for the whole of the six days during which

the experiment lasted exposed to the direct rays of the sun. The degree of damage was again measured in Rimington units, and the results illustrated graphically in Graph IV.

GRAPH IV
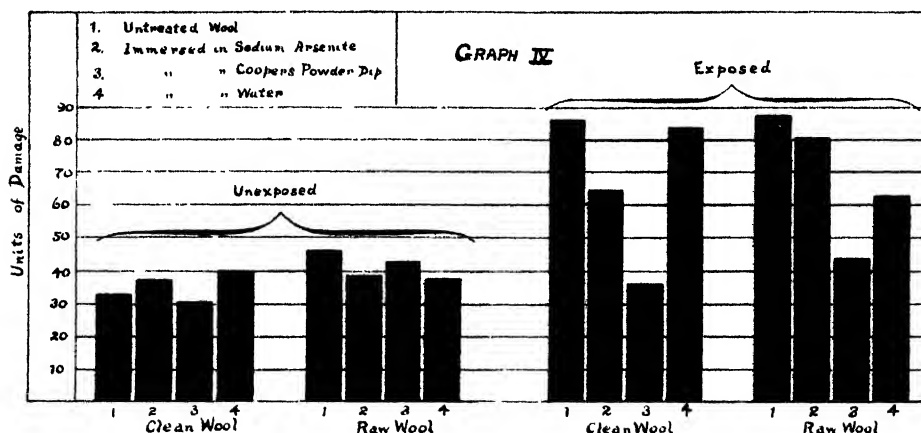
TABLE XX.

Effect of Sunlight on Wool.

Experiment.	Treatment.	Unexposed.		Exposed.	
		Clean wool.	Raw wool.	Clean wool.	Raw wool.
1.........	Not immersed—control..........	32·3	45·5	86·9	86·9
2.........	Immersed in sodium arsenite......	37·0	37·7	64·5	80·0
3.........	Immersed in Cooper's....'......	30·3	42·5	35·1	43·5
4.........	Immersed in water..............	40·0	37·0	83·3	62·5

We note that in Experiment 1, 2 and 4 the exposed wools show a much greater damage than the unexposed wools, the damage in the case of the former being about double that of the latter. In the case of Cooper's powder dip—experiment 3—exposure to sunlight seems to have had no effect. The reason for this we have already explained. These results also show that the protective influence of grease and suint is insignificant, the raw wool often showing slightly more damage than the clean fibres.

VI. SUMMARY.

The effect of the two arsenical dips, viz.: Cooper's powder dip and sodium arsenite, on the fleece has been studied from several angles. The process of dipping results in the leaching out of suint constituents, thereby influencing the hygroscopicity of the raw wool, especially at 100 per cent. humidity. The use of arsenicals as dips results in the contamination of the fleece with arsenic, some of this arsenic remaining in the fibres even after scouring. When using the

correct concentrations, the dips themselves do not cause any noticeable damage to the fibre, except that Cooper's dip tends to stain the fleece yellow by depositing its insoluble constituents. Should, however, the alkalinity of the dips rise above pH 10·3, the soundness of the fibres may be seriously affected. Under normal conditions, however, the undesirable effect of dipping on the fibre soundness must be ascribed to the process of dipping as such, and not to the use of the chemicals here studied. In-vitro studies have shown that this dipping effect in turn is merely a secondary cause of damage, the initiative and primary cause of fibre damage being the effect of the ultra-violet radiations of ordinary sunlight. In this respect arsenates and arsenites would appear to protect the fibre, rather than causing further damage, since pure water is appreciably more deleterious to such radiated wools than arsenite solutions. Cooper's dip renders additional protection to the fibre, the yellow film staining the fibres impeding the penetration of the ultra-violet rays to the fibre itself. The theoretical implications of these observations have been discussed and explained. The natural grease and suint on the fibres were not found to afford sufficient protection against the effects of sunlight. In how far these observations affect the fleece on the sheep's back must still be further investigated. It may be expected, however, that repeated dipping would aggravate this photo-chemical effect, since the staple-formation is thereby destroyed, thus exposing a greater portion of the fleece to direct radiation.

VII. REFERENCES.

BARRIT, J., AND KING, A. T. (1929). 18, The Sulphur Content of Wool. Part II. Distribution of Sulphur along the Fibre, Variation with Colour and the Effect of Exposure to Ultra-Violet Light. *Jnl. Text. Inst.*, Vol. 20, No. 7, p. T.153.

BENEDICT, S. R. (1909). The Estimation of Total Sulphur in Urine. *Jnl. Biol. Chem.*, Vol. 6, p. 363.

BONSMA, F. N. (1930). Yolk--Its Economic Importance. A Technique for the complete Analysis of a sample of greasy wool. *Wool Record and Textile World*, July 24th, p. 235.

DAVIS, J. C. (1931). A Simple, Inexpensive Quinhydrone Cell for Rapid Work. *Analyst*, Vol. 56, No. 664, p. 449.

DENIS, W. (1910). The Determination of Total Sulphur in Urine. *Jnl. Biol. Chem.*, Vol. 8, p. 401.

FOLIN, O., AND WU, H. (1919). A System of Blood Analysis. *Jnl. Biol. Chem.*, Vol. 38, p. 87.

FOLIN, O., AND SVEDBERG, A. (1930). An Improved Distillation Method for the Determination of Urea in Blood. *Jnl. Biol. Chem.*, Vol. 88, p. 77.

FOLIN, O., AND SVEDBERG, A. (1930). Micro Methods for the Determination of Non-Protein Nitrogen, Urea, Uric Acid, and Sugar in Unlaked Blood. *Jnl. Biol. Chem.*, Vol. 88, p. 85.

GREEN, H. H. (1915). The Sulphur Sheep Dips. *Dir. Vet. Res., U. of S.A.*, 3rd and 4th Repts., p. 148.

GREEN, H. H. (1918). The Micro-Titration of Arsenic. *Dir. Vet. Res., U. of S.A.*, 5th and 6th Repts., p. 539.

KERTESZ, A. (1919). Über die Wirkung der atmosphärischen Einflüsse auf Wolle und Tuche, *Textile Forschung*, Vol. I, No. 3, p. 65.

KRAIS, P., AND SCHLEBER, V. (1929). Die Erkennung chemischer Woll-schädigungen *Textile Forschung*, Vol. 11, No. 2, p. 99.

MALAN, A. I., AND V. D. LINGEN, G. W. B. (1931). Studies in Mineral Metabolism, XVI. The Micro-Determination of Some Inorganic Elements in Blood and Vegetation. *Dir. Vet. Serv. and An. Ind., U. of S.A.*, 17th *Rept.*, p. 443.

OSBORN, T. W. B., AND RAFTERY, J. D. (1932). The Ultra-Violet Content of South African Sunlight. *S.Afr. Jnl. of Sc.*, Vol. 29, p. 607.

PAULY (1904). *Zeitschr. Physiol. Chem.*, Vol. 42, p. 508. *Cf.* Rimington: *Jnl. Text. Inst.*, Vol. 21, p. T.237.

PAULY AND BINZ (1904). *Zeitschr. Farben- u. Textilindustrie*, Vol. 3, p. 373. *Cf.* Rimington, *loc. cit.*

RIMINGTON, C. (1930). A Quantitative Method for the Determination of " Soudness " in Wool and Cloth. *Jnl. Text. Inst.*, Vol. 21, p. T.237.

RIMINGTON, C. (1930). A Method for the Determination of Sulphur in Wool and Similar Materials. *Jnl. Soc. Chem. Ind.*, Vol. 49, p. T.140.

SAUER (1916). *Zeitschr. angew. Chem.*, Vol. 29, p. 424. *Cf.* Krais and Schleber (1929), *loc. cit.*

SPEAKMAN, J. B. (1931). The Action of Sodium Sulphide on Wool. *Jnl. Soc. Chem. Ind.*, Vol 50, p. T.19.

TANZER, E. (1930). Physikalisch-chemische Studien über den Feinbau des Wollhaares an mit Alkalien und Chlor behandelter Wolle. *Wissenschaftliches Archiv fur Landwirtschaft*, Vol. 4, No. 2, p. 297.

Section VII.

Animal Husbandry.

A Statistical Analysis of Growth and Carcase Measurements of Baconers.*

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I. INTRODUCTION.

For several years systematic feeding trials have been conducted with bacon pigs by officers of the Department of Agriculture at two of the Agricultural Schools, Cedara and Potchefstroom. The main objects of these trials were to investigate the suitability of different breeds of pigs and their crosses, when fed different balanced rations, for the production of first grade bacon carcases. Each separate trial was reported on by the officer who was in charge and in 1930, Romyn and others issued a report on all the trials completed at that time. These trials showed clearly that some breeds and crosses (Middle White and Berkshire) were unsuitable for bacon production. Since 1928 the work was concentrated on the following three breeds and their crosses: Large White, Large Black, and Tamworth. A large number of rations have been tested out since 1928, and the results indicated that with the balanced rations used the effect on the type of the carcases was not great. Different rations had influence on the rate of gain, thickness of back fat, and the firmness of the fat, and so indirectly may have influenced some of the carcase measurements. When the results of the different sows and boars were grouped it was found that the differences within a breed between different individuals, were much greater than the differences between crosses or between the rations that were used. Since the influence of the rations on the carcase measurements were relatively small and all being balanced and the pigs not being starved, no attention will be given to these factors in the present analysis of the data.

The object of this paper is to make a study of the growth of the baconers that were used and their carcase measurements and how these vary under different conditions such as size, degree of fatness, etc. The importance of having such data is, that when standards are to be drawn up when pig recording, for instance, is started, there is something definite on which they can be based. The same applies for standards which will be necessary when " Utility Classes " at shows are started. When bacon factories start paying out on quality basis for bacon pigs, such results will enable them to base their prices on scientific information. Hansson (1927) and Schmidt and others (1929) have made analysis of pigs recorded and tested out and their findings have been of much value, not only in the proper selection of the breeding animals, for which purpose recording and testing have been started in the first instance, but also for drawing up standards for shows 'and as information for the bacon factories when paying out on quality. In England [Davidson (1930), Duckham (1929) and Menzies Kitchen (1930), Hammond (1922)] researchers have made analyses of data, and from the information obtained standards have been drawn up.

One cannot; however, just adopt standards of another country where different conditions exist. Danish standards, for instance, would be unsuitable for South Africa and would not serve their purpose at all, since the largest percentage of pigs slaughtered in this country would not conform to them. It would be more an ideal to strive at than a standard. It is, therefore, only by analysing such material obtained from animals of about the average type in the

country from which suitable standards could be drawn up, so that most of the animals killed could conform to them. As there is improvement in the country these standards could be gradually raised. Since no outstanding breeding animals were used in these trials, it is reckoned that standards based on the results obtained will not be too severe.

Another very important aspect of such a statistical analysis of data that have accumulated during several years, is that even when results are not conclusive, one can get very useful information as regards the lines on which future investigations could be conducted. Much unnecessary work can therefore be eliminated beforehand, and the investigator knows just what to look for. This enables one also to avoid certain pitfalls since one knows already the influence and effect of certain conditions.

II. MATERIALS AND METHODS.

In the present paper an analysis is made of the growth and carcase measurements of 450-550 baconers of the Large White × Large Black (sow) and Tamworth × Large Black (sow) crosses. All the baconers were bred and fed at the two Agricultural Schools, Potchefstroom and Cedara.

Before 1929 no weights were taken of the pigs before they were weaned, but the pigs were regularly weighed after they were put in the different feeding trials. Towards the end of 1929 we started taking the birth weights of the pigs and at 4, 6, 8, and 9 weeks of age and after weaning (which took place at 8 weeks) as was done previously. When the pigs had reached weights of 190 to 210 lb. on the farm they were despatched to the Farmers' Co-operative Bacon Factory, Estcourt, Natal. They were weighed before trucking and again immediately after being unloaded at the factory. Cedara is 62 miles from Estcourt and Potchefstroom 415 miles. Those sent from Cedara were not watered or fed en route but those sent from Potchefstroom received water and some whole maize.

After the carcases had been dressed and the weights taken, each carcase was measured (the same side of each carcase) by the officer responsible for the particular trial. After that each carcase was graded by the manager of the of the factory, Mr. Welsh, and the officer. The carcases were classified into Nos. 1 and 2 " Lean Sizable ", Nos. 1 and 2 " Medium ", " Fat ", and " Overfat " grades. The following description of the grades was given by Romyn and others (1930):—

" The No. 1 ' Lean Sizable ' represents the type of side most in demand on the London market. Though well finished this type of carcase has the thinnest layer of back fat of all grades. It should also show good quality.

" A No. 1 ' Medium ' suits the north of England trade, but does not command a ready sale on the London market. This type of carcase is thicker in back fat than the No. 1 ' Lean Sizable '. In other respects they are similar.

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" The No. 2 ' Lean Sizable ' and No. 2 ' Medium ' sides represent types similar to their respective No. 1's, but lack somewhat in quality.

" The No. 1 ' Fat ' or ' Stout ' carcase is between the No. 1 ' Medium ' and ' Overfat ' in thickness of back fat. This type of carcase is becoming more and more undesirable as the demand for lean bacon increases. These carcases should also show good quality.

" The ' Overfat ' is self explanatory. Bacon is not made from this grade of side ".

In the present paper we propose working only with the following four main groups. The Nos. 1 " Lean Sizable " and " Medium " remain as they are. The Nos. 2 " Lean Sizable " and " Medium " fall in a class which will be known as " Inferior ", and the the " Stout " and " Overfat " carcases will all be put together and form an " Overfat " class.

Particulars of some of the weights and measurements that were taken are given below :—

The dressed weight consists of the two sides with the head, feet, leaf fat, kidneys, and blade bones still on and which will be removed before the sides go into the cure. After the sides had been cured and smoked, the same side of every pig was again weighed. The shoulder was then cut off between the third and fourth ribs and also weighed.

The length was taken from the front edge of the aitch bone to the front edge of the first rib. The thickness of the · back fat was taken at the thickest (shoulder) and thinnest (loin) parts and two measurements were taken over the ham. These measurements were then averaged. The depth of the side was obtained by measuring the depth behind the shoulder and at 'the flank on the outside and these were averaged. The circumference of the ham was taken where it was the greatest.

No measurements were made of the thickness of the belly, but it was judged by eye and points awarded. This was also done with the marbling of the cured sides and which were judged after the shoulders had been cut off. Points were awarded as follows: A perfect score = 10 points, very good = 9, good = 8, good medium = 7, medium = 6, and poor = 5.

All the weights were taken in pounds and the measurements in inches.

Before the measurements were taken or the grading done, samples of the back fat from one side of each carcase were taken, chopped up and rendered at the factory at a constant temperature of 110° C. The rendered fat samples were then despatched to the Chemical Division, Pretoria, where the refractive indices were determined under the supervision of Mr. Van Wyk. The refractive indices are given at 40° C.

III. ANALYSIS OF THE DATA.

Part A.—Growth.

1. *Preweaning Results.*

Since the data collected of the pigs before they were weaned, are limited, no detailed analysis is possible. Some of the averaged results will therefore only be given.

For 39 farrowings the average gestation period was 113·7 days for Large Black and Large White sows. This is nearly 1 day less than found by Carmichael and Rice (1920), their figure being 114·6 days for 7 different breeds. Of the 39 farrowings the shortest gestation period was 105 days and the longest 118 days, 77 per cent., however, being from 112 to 115 days.

The average birth weight of 494 pigs born alive was 2·89 lb. There were 271 males and 223 females and their birth weights were 2·99 lb. and 2·78 lb. respectively. 8·6 per cent. and 8·3 per cent. of the males and females respectively, were born dead. When the pigs are grouped according to the number born per litter, then there is a continuous decrease in the birth weights of both sexes as shown in table 1 :—

TABLE 1.—*Birth Weights of Pigs.*

Litter Size.	6–8.		9–11.		12–14.		15–17.	
Sex..................	M.	F.	M.	F.	M.	F.	M.	F.
No. of pigs.................	38	26	108	103	79	64	22	20
Average weight, lb...........	3 5	3·17	3 08	2 75	2 69	2·67	2·34	2·21

Carmichael and Rice (1920) found the same, but the birth weight of the males (2·59 lb.) was only ·08 lb. more than that of the females (2·51 lb.). Litters of less than 8 pigs at birth were heavier (2.67 lb) than the average for all the pigs (2·55 lb. for 5,188 pigs). The litters of more than 8 pigs at birth had an average weight of 2·47 lb. Eaton (1932) states that in the case of guinea pigs litter size and length of gestation period determines more than 60 per cent. of the birth weight. Haines (1931), also working with guinea pigs, found the same influence and also that mortality increased with increase in litter size, and in the rabbit Hammond (1925) states this to be the case also.

The birth weight being correlated with the later weights, as shown by Eaton (1932), and the mortality also being higher among the lighter pigs [Wenck (1931) and Haines (1931)], it is important to strive at heavier pigs at birth. This, however, is not the only factor to be considered since for economic pig production a sow should not raise less than 8 pigs per litter, so that one should find out what the optimum number is when these two factors are taken together. The weight of the litter at 4 or 8 weeks of age is quite a good indication, although the milk supply of the sow plays an

important part. For commercial purposes, however, this is what counts. In table 2 an indication is given of the influence of the litter size on the total litter weight at 8 weeks of age.

TABLE 2.—*Influence of Litter Size on Litter Weight at 8 Weeks.*

Litter Size.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
No. of litters..	3	3	3	5	7	8	7	7	2	1	1	1
No. of pigs weaned......	14	20	20	34	56	59	63	63	18	6	9	12
No. of pigs weaned per litter.	4·7	6 7	6·7	6·8	8·0	7·4	9·0	9·0	9·0	6·0	9·0	12·0
Total litter wt. at 8 weeks, lb.	173	227	240	248	256	225	309	264	243	179	215	336

Although not quite consistent on account of the small number of observations, there is nevertheless an increase at first with an increase in litter size up to 12 pigs per litter, when the weights start decreasing. Investigators working with much larger numbers have found the same thing. The percentage weaned per litter decreased with increase in litter size, but the decrease was found to be much more rapid with litters of more than 12 pigs at birth. Wild (1929), for instance, got the following percentages: when all pigs born (including those born dead) were taken, the average for all taken together being 72 per cent. weaned. In litters of over 17 pigs, only 35 per cent. were weaned, 12 to 16 pigs, 60 per cent., and 8 to 11 pigs, 80 per cent., and those with less than 8 pigs, 85 per cent. Wenck's (1931) results also showed a very high mortality for litters above 13 pigs at birth, and he comes to the conclusion that one should strive at 8 to 12 pigs per litter with as high a weight at 4 weeks of age as possible. Buchanan Smith (1930) reckons that the ideal litter size is from 10 to 12 pigs at birth.

Of the pigs that were born alive, 81 per cent. of the males and 76 per cent. of the females were weaned. Whether the difference in birth weight caused this difference is difficult to say. Under the same conditions one would expect that more females would reach weaning age than males. From Wenck's (1931) and Haines' (1931) investigations it seems quite likely that the difference in the percentage weaned of the two sexes may have been influenced by the difference in birth weight, since the mortality among the lighter pigs is higher than among the heavier ones. Haines analysing the data of 30,000 guinea pigs, found that of those born alive 1 per cent. more males than females were raised to weaning.

The sows and the idividual pigs were only weighed at 14 days intervals after the pigs were 4 weeks old. No data are therefore available of the loss of weight of the sows from farrowing up to 4 weeks after farrowing. From 4 to 8 weeks after farrowing the sows lost on an average 27 lb. per sow. It also appears that the heavier the total litter weight at weaning, the larger is the loss in weight of the sow as indicated in table 3.

TABLE 3.—*Loss in Weight by the Sows before Weaning.*

Total litter weight, ℔..........	180	200	220	240	260	280	300	320	340
No. of sows....................	4	2	6	8	10	2	3	1	5
Loss in live weight, ℔..........	8	9	16	30	30	28	18	59	44

Huszti (1930) regularly weighed 22 Mangalicza sows every 2 weeks after farrowing and he found that during the first 4 weeks the sows lost 13·2 Kg. During the 5th week their weights remained constant and from the 6th week started to gain in live weight, so that at 9 weeks the total loss was 10·6 Kg. per sow. In the abstract no litter weights are given, but the average daily gain of the pigs up to weaning was only 128 gm. or ·28 lb., whereas those under investigation gained ·52 lb. The Mangalicza sows are probably poor milkers and therefore start gaining in weight at 5 weeks.

2. *The Weights at Different Ages.*

When pig recording is started then it is important to have a standard growth curve by which to compare the pigs coming from all parts of the country. As conditions differ so much in different countries, the standard of one country may not be applicable to another country. In table 4 and Diagram 1 the weights of pigs are given at different ages up to 21 weeks of age. After that period the quickest growers were killed, so that the average weights after 21 weeks of age will not be representative. For the sake of comparison the results of other investigators are also included.

DIAGRAM I.
Normal Gains of Baconers.
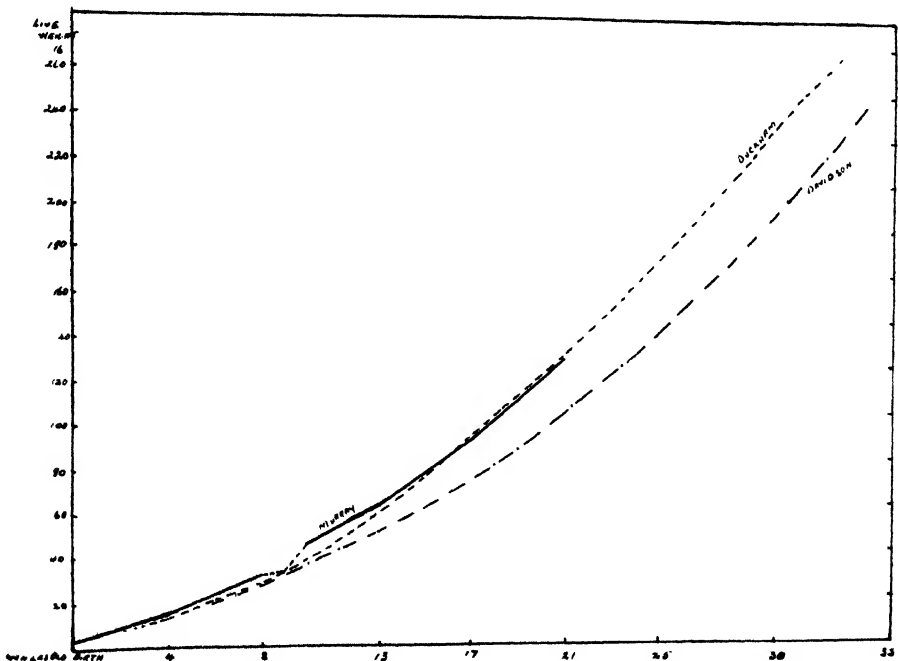
TABLE 4.—*Weights in lb. at Different Ages.*

Age in Weeks.

	Birth	2.	4.	6.	8.	9.	10.	13.	17.	21.	25.	29.	33.	37.
Murray......	2·89	—	15 5	23 1	32·2	33·9	46·3	62 0	91·3	128 2	—	—	—	—
Duckham.... (1929)	2·5	9·0	14·0	21·0	28 0	33 0	39 0	59·0	93 0	129 0	170	215	261	302
Davidson.... (1930)	—	—	—	20 0	29·0	33 5	37·8	51·5	74·0	104·0	139	179	225	—
Stahl (1930)..	2·7	7 5	12·9	20·1	30·6	36 5	43·3	—	—	—	—	—	—	—
Schmidt..... (1929)	—	—	—	—	—	—	—	74 8	112·2	152·8	191·4	—	—	—

From the above table it is seen that our figures agree well with those of Duckham (1929) and which were used as the standard in the East Anglican Pig Recording Scheme. Our birth weights are higher than those of the others and also higher than the figure obtained by Carmichael and Rice (1920) which was 2·55 lb. Up to 15 weeks Duckham's curve is slightly below ours and after that slightly above. Stahl's (1930) results do not show a big difference either. Those of Schmidt (1929), which were obtained from the testing station, are much higher than the rest. From the age of 10 weeks onwards, Davidson's (1930) figures are lower than the rest. He reckons that Duckham's figures are on the high side for English conditions and that too large a percentage of sows are consequently penalised. As regards the weaning standard (8 weeks of age), the different figures correspond well and fall round 30 lb. per pig. The upper limit, i.e., the age when ready for bacon, however, show rather big differences. It would appear that our curve should be taken as the standard and only when it appears to be too high for the country should it be lowered.

Individual Variability in Weight.—An analyses has been made of the variability in the weights of the pigs at the different ages, the weights of the barrows and gilts being kept separately. The two measures of variability, viz., the standard deviation (S.D.) and the coefficient of variability (C.V.), have been determined and the results shown in table 5.

TABLE 5.—*Variability at Different Ages.*

	Age in Weeks.	Birth.	4.	6.	8.	9.	13.	17.	21.
Males.	No. of pigs......	271	197	194	193	68	271	269	270
	Mean weights, lb.	3·09	16·0	23·7	34·5	34·4	61·8	92·1	132·1
	S.D., lb.........	·729	3·62	4·89	9·0	7·78	20·25	31·24	44·29
	C.V., per cent....	23·23	22·7	20·65	26·1	22·63	32·76	33·91	33·53
Females.	No. of pigs......	223	164	161	157	69	211	210	210
	Mean weights, lb.	2·85	15·2	22·8	32·0	33·5	62·1	90·3	125·3
	S.D., lb.........	·808	3·02	4·14	6·46	5·66	19·63	29·3	39·25
	C.V., per cent....	27·87	19·83	18·2	20·18	16·89	31·61	32·45	31·32

Except for the birth weights (only those born alive are included), the standard deviation and the coefficient of variability are higher for the males than for the females. Hammond (1932) found that the standard deviation of wethers was greater than that of the ewes except at 1 week of age, at which age the ewes were the heavier, whereas the wethers were the heavier at the other ages. From the results in table 5 it does not appear as if there is the very close relationship between live weight and standard deviation as was found for sheep by Hammond. As the live weight increases, however, so does the standard deviation or actual variability.

The coefficient of variability does not show the same regular tendency as the standard deviation. At birth the coefficient of variability is high and decreases up to 6 weeks of age. At 8 weeks there is quite a marked increase for the males and only a slight increase for the females. At 9 weeks it has decreased again for both sexes. From 13 weeks of age onwards the standard deviations and the coefficients of variability are high, the former continually increasing and the latter remaining about the same. Hammond (1922) found that the maximum variability in weight occurred at 7 months and this corresponds with the maximum daily gain. Our figures are rather higher than those determined by him, except his maximum (33·1) which is about the same as found from 13 to 21 weeks in table 5. Wentworth and Lush (1923) found the maximum variability to be at 5 months and which decreased after that age. From table 5 it appears that the variability will decrease rather than increase after 21 weeks, so that the maximum was reached at 17 weeks or 4 months. This is therefore at an earlier age than found by Hammond and Wentworth and Lush.

When the weekly gains are taken from birth to 21 weeks of age instead of the live weights, then the coefficients of variability closely follow the weekly rate of gain in the preceding period as shown below. The differences in the rates of gain made by the pigs would appear to be the cause of the variations in the coefficients of variability at the different ages. Only the pigs that were weighed at 9 weeks were taken to get the gain between 8 and 9 weeks and hence the difference from table 5.

Weeks	Birth.	— 4	— 6	— 8	— 9	—13	—17	—21
Males. Weekly gains, lb.	.....	3·2	3·6	5·4	4·3	6·8	7·6	10·0
Males. C.V., per cent...	.....	22·7	20 6	26·1	22·6	32·7	33·9	33·5
Females. Weekly gains, lb.	.....	3·1	3·8	4·6	3·2	7·1	6·9	8·7
Females. C.V., per cent...	.....	19·8	18·2	20·2	16·9	31·6	32·4	31·3

Hammond (1932) discusses the findings of different investigators as regards the difference in the coefficient of variability of the sexes. Darwin concluded that males were more variable and Havelock Ellis (1914) and Karl Pearson had a controversy as to whether man or woman is the more variable. Pearson states that woman is slightly more variable than man, while Ellis concludes that man is the more variable. When no allowance is made for the rate of gain or the difference in live weight, then the male (barrow) is more variable than the female (gilt) as shown in the present investigation.

To see the variability of the sexes at the different ages, when the factor of difference in rate of gain had been eliminated, table 6 has been prepared. The males and females have been divided into fast (* gaining 1·5 lb. and above per pig per day), medium-fast (gaining 1·2 to 1·49 lb. per pig per day), and slow (gaining 1·19 lb. and below per pig per day) growers.

TABLE 6.—*Variability of Fast, Medium-fast, and Slow Growers.*

		1·5 and above.			1·2–1·49.				1·19 and below.				
Average daily gain—lb...													
Age in weeks		13	17	21	13	17	21	25	13	17	21	25	29
Males.	No. of pigs	75	75	75	98	98	98	94	97	97	97	95	93
	Mean weight—lb	77·4	123·3	173·4	65·2	98·2	137·2	183·9	46·9	64·7	90·3	120·0	162·2
	S.D.—lb	17·6	24·3	28·3	18·8	26·2	26·6	24·1	10·7	15·7	19·7	26·4	31·0
	C.V.	22·7	19·6	16·3	28·8	26·6	19·4	13·1	22·9	24·2	21·8	21·9	19·1
Females.	No. of pigs	40	40	40	76	76	76	64	95	94	94	94	91
	Mean weight—lb	77·5	119·8	168·8	70·2	103·5	144·1	187·3	49·1	67·2	91·8	119·4	152·2
	S.D.—lb	18·6	22·1	24·6	18·7	21·7	24·9	19·0	10·8	16·3	20·8	26·0	32·6
	C.V.	23·9	18·5	14·6	26·7	21·0	17·3	10·1	22·0	24·3	22·6	21·7	21·4

*The gains have been determined from the commencement of the trials until the pigs were slaughtered.

For the fast and medium-fast growers the standard deviations and coefficients of variability are consistently lower for the females except at 13 weeks for the fast growers, where the variability of the females is slightly higher than that of the males. As for the slow growers there is no consistent difference between the two sexes. Diagram II shows the trends of the variability for the different groups and sexes. The standard deviation of the fast growing males is still increasing at 21 weeks. The females show a smaller increase from 13 to 21 weeks. The medium fast growers show a decrease from 21 weeks to 25 weeks for both sexes and the slow growers a continual increase. Whereas the standard deviations of the fast and medium-fast groups at the different ages, do not show large or consistent differences, the coefficients of variability are decidedly lower for the fast growers. The coefficients of variability of the fast and medium-fast growers show a continual decrease from 13 weeks of age onwards, while those of both sexes of the slow growers remain fairly constant, showing little difference at 13 and 29 weeks. This, therefore, agrees with what Hammond (1932) says, that: " animals, after they have had their growth forced by high feeding, should be on the whole less variable (coefficient of variability) than those which have previously

had their growth retarded and are still actively growing ". As will be shown in another section the slow growers make much more rapid growth later on, and consequently show little change in the co-efficients of variability in comparison with the fast and medium-fast growers.

DIAGRAM II.

Coefficients of Variability and Standard Deviations of Fast, Medium and Slow-growing Barrows and Gilts.
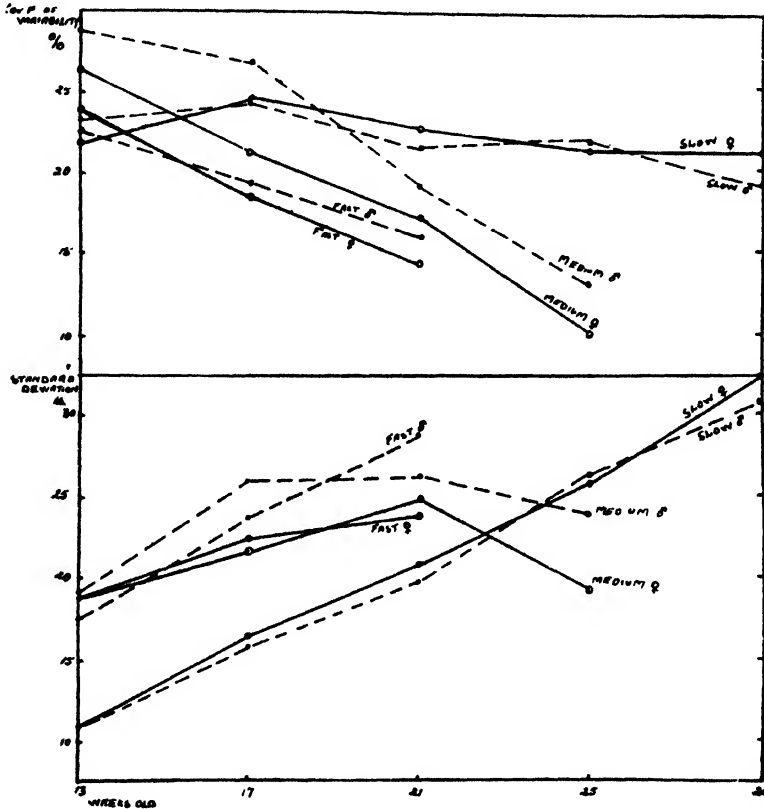
Correlation between Weights at Different Ages.—Wild (1929) and Wenck (1931) found that the pigs which were the heaviest at the young stages were also the heaviest at slaughter. The former took the earliest weights at 10 weeks and the latter at 4 weeks of age. Ham-mond (1932) got the same with sheep. The correlation became larger the shorter the period between the end weight and the earlier weights. Schmidt and others (1929), however, found no relation between the weights at 4 weeks and the weights at slaughter in the case of pigs. In this paper the weights at 21 weeks have not been correlated with those of the pigs before they were weaned since only recently full preweaning particulars have been kept of the pigs used in the different feeding trials. Correlation coefficients have, there-fore, only been determined between the weights at 4 and 8 weeks and between the weights at 13 and 21 weeks, the number of animals

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available being 338 and 479 respectively. The correlation coefficient between the 4 and 8 weeks ages is $+0.76 \pm .02$ and between the 13 and 21 weeks ages $+0.89 \pm .01$. Although the period is twice as long in the second case, the correlation coefficient is nevertheless significantly larger than in the first case. An important factor which probably contributed to this difference, is the milk supply of the sows. Before weaning the inherent ability of a pig for growing and the milk supply of the sow are the determining growth factors. The pigs under investigation received as much feed as they could consume when put in the trials, so that only the one factor had influence on the growth after the pigs were weaned. According to Olofsson and Larsson (1930) the sow's milk still constitutes 40 per cent. of the pig's diet at 8 weeks, so that the milk supply of the sow is still an important factor in this instance. A sow may be a heavy milker at the beginning of her lactation but the milk supply may then drop very suddenly, as is very often found with cattle. Pigs suckling such sows may start off well but lose again some of the advantage before they are weaned and in this way resulting in less strong correlations between weights at different ages before weaning than after weaning. This is another strong argument in favour of weighing pigs at 8 weeks (weaning) of age instead of at 3 or 4 weeks of age for recording purposes. One then has a measure of what a sow is capable of handing over to the farmer and at the same time has an indication of what the pigs will be able to do after weaning up to the slaughter age.

The actual weights of the pigs at 4 weeks and the weights of the same pigs at 8 weeks and weights of pigs at 13 weeks and weights at 21 weeks, are given below :—

TABLE 7.—*Relation between Weights at Different Ages.*

Relation between weights at 4 and 8 weeks.			Relation between weights at 13 and 21 weeks.		
No. of Pigs.	Weights at 4 weeks.	Weights at 8 weeks.	No. of Pigs.	Weights at 13 weeks.	Weights at 21 weeks.
	lb.	lb.		lb.	lb.
3............	8	17·7	6............	20	71·3
3............	9	20·7	45............	30	77·8
14............	10	22·2	101............	40	92·4
11............	11	26·5	107............	50	111·8
28............	12	26·6	66............	60	140·1
28............	13	27·3	44............	70	160·4
28............	14	30·5	51............	80	170.7
32............	15	30·9	39............	90	183·0
56............	16	32·4	21............	100	197·8
31............	17	35·9			
29............	18	36·8			
16............	19	40·3			
26............	20	38·2			
8............	21	41·9			
5............	22	40·2			

3. *Rate of Gain.*

In this section the intention is to see how the rate of gain changes for the different groups (fast, medium-fast, and slow growers), and also how the gains are influenced by the sexes. It has already been shown that the males are slightly heavier than the females, but to get a better idea of the relationship between the gains of the sexes, the weights of the females have been taken as 100 at the different ages and those of the males expressed as ratios thereof. The actual and relative weights are given in table 8, and diagram III shows how the relative weights change.

TABLE 8.—*Relative and Actual Weights of Barrows and Gilts at Different Ages.*

	Birth.	Weeks Old.							
		4.	6.	8.	9.	10.	13.	17.	21.
Relative wts. barrows	107·6	104·1	104·1	102·1	99·6	99·9	99 7	102·1	104·2
Actual weights, lb.—									
Barrows..........	2 99	15·7	23·5	32·5	33·8	46 3	61·9	92·1	130·6
Gilts........... ..	2 78	15 1	22·6	31·8	34 0	46 32	62 1	90·2	125 2
Average..........	2 89	15·5	23·1	32·2	33·9	46·31	·62·0	91·3	128 2

DIAGRAM III.
Relative Weights of Barrows and Gilts.
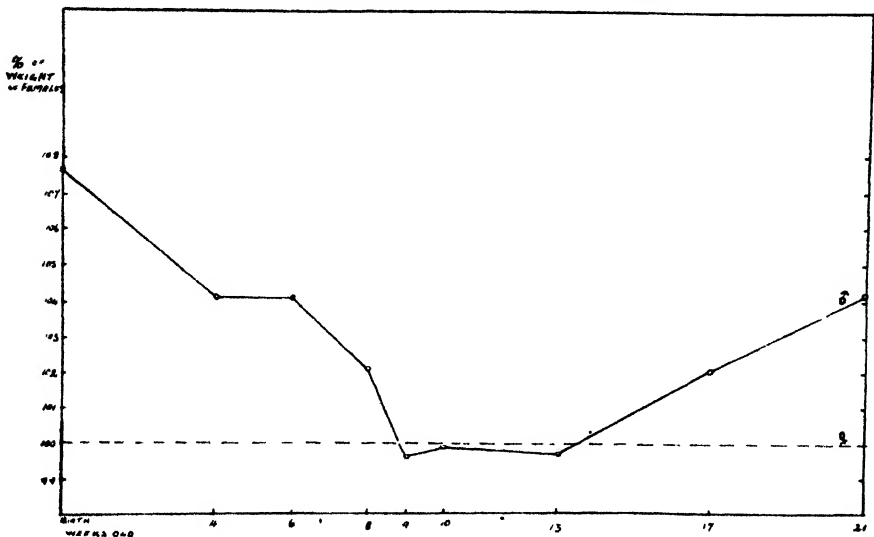
The trend of the relative growth of the barrows and gilts is quite definite. At birth the males have appreciably higher weights than the females. This difference decreases until it is actually lower from the 9th to the 13th week and then the males start increasing

again in weight. The drop in the relative weights of the males is probably due to the effect of castration and weaning. Between 3 and 4 months when the females reach puberty their growth is also retarded. Wilkens (1929) also found that the difference in weight becomes greater in favour of the males with an increase in age.

The 271 males and 210 females have been divided into fast, medium-fast and slow growers to see how the gains of the three groups are affected at the different ages. The sexes are divided up as follows in the three groups: —

	Fast growers (gaining 1·5 ℔. daily and over).	Medium-fast growers (gaining 1·2–1·49 ℔. daily).	Slow growers (gaining 1·19 ℔. and less).
Males, per cent.....	27·7	36·5	35·8
Females, per cent..	19·0	36·2	44·8

The males have only 8·1 per cent. more slow than fast growers, whereas the females have 25·8 per cent. more.

The gains made from birth up to 90 days of age have been obtained and then the gains made every 28 days for the individual pigs in the three groups. The gains made up to 90 days of age have been taken as 100 and the subsequent gains expressed as percentages of this. The results are given in table 9 and shown in diagram IV.

TABLE 9.—*Relative and Actual Gains of the Fast, Medium Fast and Slow Growers.*

	No. of Pigs	Age in Days.						
		90.	118.	146.	174.	202.	230.	258.
Males.								
Fast growers—								
Actual gains—℔..	75	·858	1·544	1·881	1·932	—	—	—
Relative gains....		100	180	219	225	—	—	—
Med-fast growers—								
Actual gains—℔..	99	·725	1·137	1·434	1·687	1·871	—	—
Relative gains....		100	157	198	233	258	—	—
Slow growers—								
Actual gains—℔..	97	·52	·624	·909	1·09	1·477	1·552	1·85
Relative gains....		100	120	175	210	284	298	356
Females.								
Fast growers—								
Actual gains—℔..	40	·861	1·525	1·75	1·804	—	—	—
Relative gains....		100	177	203	210	—	—	—
Med-fast growers—								
Actual gains—℔..	76	·776	1·199	1·436	1·588	1·685	—	—
Relative gains....		100	155	185	205	217	—	—
Slow growers—								
Actual gains—℔..	94	·546	·627	·888	1·009	1·243	1·414	1·913
Relative gains....		100	115	163	185	228	259	350

DIAGRAM IV.

Relative Gains of Fast, Medium and Slow-growing Barrows and Gilts.
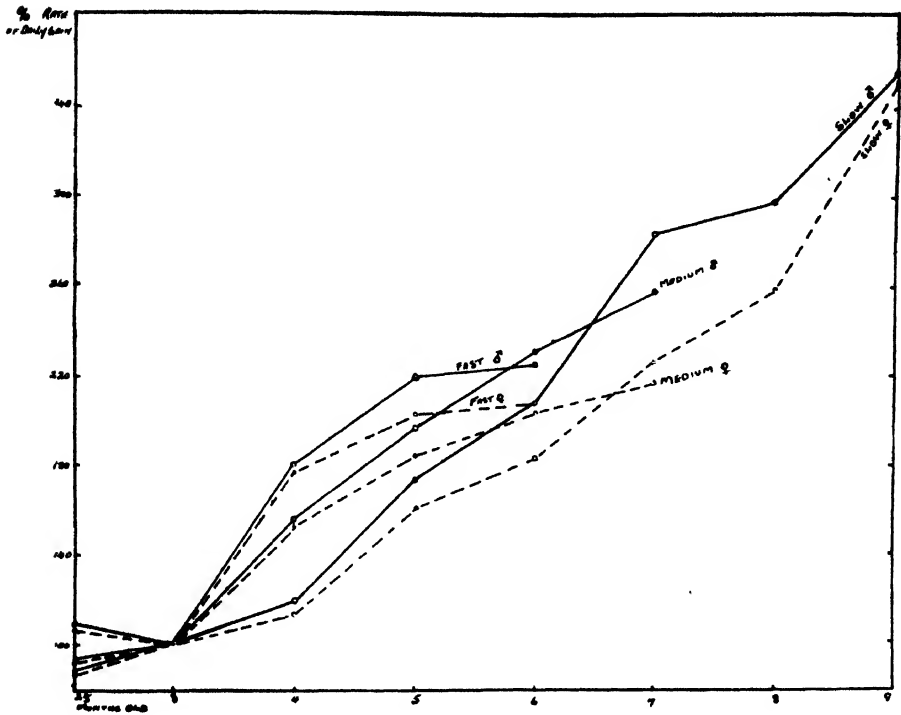
The pigs came into the feeding trials at an average age of 10 weeks. The weights at 10 weeks decrease for the three groups, the females being slightly heavier. Even at 90 days the females have made better gains than the males within the different groups. At 118 days (4 months) the fast growing males are showing better gains, but in the other two groups the females are still better. It is, however, between the ages of 4 and 5 months that the males really begin to outstrip the females. The differences between the sexes in the three groups are shown better by the relative gains. The fast growing groups show a rapid rise in the relative gain during the first 28 days. The steepness of the curves decrease from the 4th to the 5th months and then the relative gains remain about the same from the 5th to the 6th months. The medium-fast growing groups have fairly high relative gains during the first 28 days and then there is also a decrease in the steepness of the curves but not so much as in the first group. In the slow growing group the increase is not much during the first 28 days and the steepness of the curves increases up to 5 months when there is a slight decrease in the steepness but an increase again from the 6th month. At the end (8 to 9 months) the females show a very sharp rise ending up near to the males.

All three groups show little differences between the sexes up to 4 months. From 4 months the differences in relative gains of the males start to show increasing gains over the relative gains of the

females and at 5 months the fast-growing group show the largest difference between the sexes. The differences of the other two groups are about the same. At 6 months the difference of the fast group has not increased much but has continued to increase in the other groups, the medium-fast group showing the largest difference between the sexes. Both the remaining groups continued to increase in the differences, but the slow group shows the largest difference in favour of the males at the age of 7 months. The curve of the slow growing males shows an irregularity between 7 and 8 months, but shows definitely that the difference in gains between the sexes is becoming less and is nearly the same at 9 months. The curves of the other two groups have shown the same tendency just before they stopped and would quite likely have approached each other if data for longer periods had been available. These differences, which first increase and afterwards decrease again at different ages for the different groups, seem to be caused by the coming on heat of the females at different ages, and that the slow growers are influenced more by these heat periods than the fast growers. As the animals become older they seem to be less affected by heat and hence the converging of the curves of the sexes after a time.

An interesting study could be made on these lines by castrating half the males and half the females and then giving all the same management and feeding until they are mature.

PART B.—FACTORY RESULTS.

1. *Average Results of the two Crosses.*

Before analysing the data of the pigs of the two crosses together, it is necessary to see whether they do not show any marked differences. Hansson (1927) analysed the data of the pigs of the Swedish Landrace and Large Yorkshire coming from the testing station, but he did not find any marked breed differences. Schmidt and others (1929) again worked with the German Landrace and the Edelschwein and they did not find any marked breed differences either. In both the Swedish and German results it came out very clearly that there were much larger differences within the breeds than there were between the breeds. The individual boars and sows exerted greater influence on the progeny than the breed they belonged to. The two Swedish breeds and two German breeds mentioned are more or less of the same type. This is also the case with the three breeds used, to get the two crosses on which this work was done, so that one will not expect any marked diffrences with the data we are presenting. The average results of the two crosses are given in table 10 along with the relative weights and measurements. The length, which is an early maturing measurement, has been taken as 100 and the weights and measurements expressed as percentages thereof.

TABLE 10.—*Average and Relative Weights and Measurements of the two Crosses.*

	Actual Measurements.		Relative Measurements.	
	Tamworth X Large Black.	Large White X Large Black.	Tamworth X Large Black.	Large White X Large Black.
No. of pigs.....................	156	295	—	—
Initial age—days...............	71·1	71·3	—	—
Final age—days................	188·0	189·4	—	—
Initial weight—lb...............	46·3	48·9	159	170
Final weight—lb...............	194·8	196·2	669	682
Factory weight—lb............	178·5	180·9	—	—
Percentage of farm weight......	91·6	92·2	—	—
Carcase weight—lb............	148·5	146·0	510	507
Percentage of farm weight......	76·2	74·4	—	—
Weight of cured side—lb........	50·9	51·6	175	179
Percentage of farm weight (2 sides)	52·3	52·6	—	—
Weight of shoulder—lb.........	15·3	15·6	53	54
Percentage of whole side........	30·1	30·2	—	—
Average daily gain—lb.........	1·306	1·31	—	—
Length of side—inches..........	29·1	28·8	100	100
Depth at shoulder—inches......	16·35	16·39	56	57
Depth at flank—inches.........	15·85	15·69	55	55
Average—inches...............	16·1	16·04	55	56
Ratio— $\dfrac{Shoulder}{Flank} \times 100$.....	103·2	104·5	—	—
Circumference of ham—inches...	23·34	24·06	80	84
Back fat (thickest) in inches.....	2·03	2·19	7·0	7·6
Back fat (thinnest) in inches.....	1·347	1·277	4·63	4·44
Average—inches...............	1·511	1·532	5·19	5·32
Evenness back fat—per cent....	66·4	58·3	—	—
Thickness of belly per cent.....	81·7	74·3	—	—
Marbling per cent.............	69·5	70·0	—	—
Refractive index at 40°C.......	1·4596	1·4597	—	—
Classification { I.L.S.—per cent.........	42·9	49·8	—	—
I.M.—per cent...........	42·9	20·0	—	—
O.F.—per cent...........	9·0	9·8	—	—
Inferior—per cent........	5·2	20·3	—	—

The gains made by the pigs of the two crosses are the same and the pigs were slaughtered at the same average live weights. The length of side, the thickness of the back fat and the thickness of the belly were somewhat better for the Tamworth cross than the Large White cross and this affected the grading to an extent, the first mentioned cross having 15 per cent. more first grade carcases than the second cross. The Large White cross had slightly better hams. The relative measurements do not show any marked differences either.

To see how different boars can influence the measurements, the average results of two Large White boars and one Tamworth boar are given in table 11. The results of the two Large White boars were obtained from the same 7 sows and 4 of these were used with the Tamworth boar. These pigs were used at the School of Agriculture, Cedara.

The average live weights of the pigs from the second and third boars are the same, but the weight of the first one is about 13.5 lb. higher. The average daily gain of the second boar is the best and the first and third are about the same. The progeny of the Tamworth

boar were much shallower than those of the Large White boars and also had poorer hams. The Tamworth boar had the thinnest back fat but the bellies were nevertheless the same as for the boar with the thickest back fat, but the marbling of the Tamworth boar was not so good. The first and third boars have the same percentage of first grade carcases (93 per cent.), but the third one has more 1 Lean Sizable carcases (53·6 per cent. in comparison with 37·5 per cent. of the first boar), but this difference was rather caused by the difference in weight than the difference between the boars. The second boar, however, has only 50 per cent. of first grade carcases and 27·8 per cent of the carcases are over fat.

TABLE 11.—*Average Results of three Boars.*

Boar.	Actual Measurements.			Relative Measurements.		
	L.W. 1.	L.W. 2.	Tam. 3.	L.W. 1.	L.W. 2.	Tam. 3.
No. of sows....................	7	7	4	—	—	–
No. of pigs....................	56	54	28	—	—	—
Initial weight—lb.............	46·3	42·3	46·8	159	150	160
Final weight—lb..............	208·5	195·1	194·9	718	690	666
Factory weight—lb............	196·6	183·8	180·2	—	—	—
Percentage of farm weight.....	94·3	94·2	92·5	—	—	—
Carcase weight—lb............	161·7	154·8	143·8	557	548	491
Percentage of farm weight.....	77·6	79·7	73·8	—	—	—
Age—days....................	175·6	155·4	165·1	—	—	—
Average daily gain—lb.........	1·188	1·256	1·181	—	—	—
Length of side—inches........	29·04	28·27	29·27	100	100	100
Depth at shoulder—inches......	16·79	16·8	16·15	58	59	55
Depth at flank—inches........	16·64	16·29	15·28	57	58	52
Average—inches..............	16·72	16·55	15·72	58	59	54
Ratio—per cent..............	100·9	103·1	105·7	—	—	—
Circumference of ham—inches..	24·23	24·23	22·83	83	86	78
Back fat (thickest) —inches.....	2·155	2·169	1·925	7·4	7·7	6·6
Back fat (thinnest) —inches.....	1·295	1·395	1·232	4·5	4·9	4·2
Evenness—per cent...........	60·1	64·3	64·0	—	—	—
Thickness of belly—per cent....	81·4	77·2	76·1	—	—	—
Marbling—per cent...........	69·1	78·7	66·8	—	—	—
Classi-fication { I.L.S.—per cent........	37·5	24·1	53·6	—	—	—
I.M.—per cent..........	55·4	25·9	39·3	—	—	—
O.F.—per cent..........	3·6	27·8	—	—	—	—
Inferior—per cent.......	3·6	22·2	7·1	—	—	—

L.W. Boar 1.—Fairholm Vanguard 7th, Reg. No. 183. Age 2 years.

L.W. Boar 2.—Grantham Tom 4th, Reg. No. 278. Age 3 years.

Tamworth Boar 3.—No particulars available.

From tables 10 and 11 it is also clear that the differences due to the different crosses are quite small when compared with the differences of the progeny of the different boars. Such marked differences were also found between different sows. These small differences between the two crosses, therefore, entitle one to treat the data of the two together. In the rest of the paper no difference will be made between the results of the two crosses.

2. *Measurements of the Different Grades.*

As the grading is done by sight and no measurements are taken to determine the grade of the carcase, it will be of interest to see in which respects the measurements of the different grades differ. The actual and relative measurements are given in table 12.

TABLE 12.—*Average Measurements of the different Carcase Grades.*

Grade.	Actual Measurements.					Relative Measurements.				
	1LS.	1M.	O.F.	2LS.	2M.	1LS.	1M.	O.F.	2LS.	2M.
No. of pigs.........	194	126	43	74	14	—	—	—	—	—
Initial age—days....	70·5	70·5	71·3	72·2	68·3	—	—	—	—	—
Final age—days.....	194·4	184·7	174·1	192·6	181·3	—	—	—	—	—
Initial weight—lb....	48·4	48·0	49·1	46·8	44·5	167	165	171	164	153
Final weight—lb....	193·2	200·8	199·2	190·5	198·9	668	690	694	666	686
Factory weight—lb....	177·0	186·4	185·1	174·1	181·6	—	—	—	—	—
Per cent. of farm wgt.	91·6	92·8	92·9	91·4	91·3	—	—	—	—	—
Carcase weight—lb..	144	150·8	151·4	139·5	145·9	498	518	527	488	503
Per cent...........	74·5	75·1	76·0	73·2	73·4	—	—	—	—	—
Weight cured side—lb.	49·7	52·7	54·8	50·5	53·0	172	181	191	177	183
Per cent. (2 sides)..	51·4	52·5	55·0	53·0	53·3	—	—	—	—	—
Weight shoulder—lb.	15·2	15·9	16·7	15·4	15·7	53	55	58	54	54
Per cent. of side....	30·6	30·2	30·5	30·5	29·6	—	—	—	—	—
Average daily gain—lb.	1·234	1·377	1·527	1·244	1·404	—	—	—	—	—
Length— inches......	28·94	29·12	28·72	28·59	29·0	100	100	100	100	100
Depth at shoulder—inches	16·19	16·62	17·0	16·05	16·68	56	57	59	56	58
Depth at flank—ins.	15·55	16·13	16·4	15·31	15·93	54	55	57	54	55
Average —inches.....	15·87	16·38	16·7	15·68	16·31	55	56	58	55	56
Ratio..............	104·1	103·0	103·7	104·8	104·7	—	—	—	—	—
Circumference ham—inches	23·7	23·9	24·16	23·8	23·9	82	82	84	83	82
Back fat (thickest)—inch	2·03	2·222	2·502	2·157	2·342	—	—	—	—	—
Back fat (thinnest)—inch	1·17	1·39	1·642	1·225	1·386	—	—	—	—	—
Average—inch.......	1·411	1·634	1·818	1·492	1·675	4·9	5·6	6·3	5·0	5·8
Evenness—per cent..	57·6	62·6	65·6	56·8	59·2	—	—	—	—	—
Thickness of belly—per cent.	77·0	83·8	82·3	64·5	68·1	—	—	—	—	—
Marbling—per cent..	68·2	70·4	76·7	67·8	75·0	—	—	—	—	—
Refractive index.....	1·4598	1·4594	1·4593	1·4597	1·4596	—	—	—	—	—

The two grades 2 Lean Sizable and 2 Medium include pigs which may have the correct thickness of back fat to fall in one of the first grades but may be too short, or long enough but " unfinished ", or both length and the back fat may be alright but the belly may be

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too thin. The average measurements of these mentioned factors are therefore not of the same value as those of the other three grades. The three grades 1 L.S., 1 M. and O.F. all have the same initial weights, but the other two grades were lighter at the commencement of the trials. The final weights are the lowest for the two L.S. grades, whereas the other three grades are about the same. The final ages show large differences, being highest for the 1 L.S. grade (194 days) and lowest for the O.F. grade (174 days), the 1 M. grade being in-between. The two inferior grades correspond to the respective first grades. The average daily gains are in the same order as the final ages of the five grades.

The loss in weight on the journey is lowest for the 1 M. and O.F. grades and the other three grades had larger losses which are about the same. The carcase percentage shows an increase from 74·5 per cent. for the 1 L.S. grade to 76·0 per cent. for the O.F. grade, the 1 M. grade being 75·1 per cent. It is rather striking that the carcase percentages for the two inferior grades (2 L.S. and 2 M.) are lower than those of the other three grades, although both have thicker back fat than the 1 L.S. grade and the 2 M. being fatter than the 1 M. as well. The percentage of cured sides to farm live weight increase from 51·4 per cent. for the 1 L.S. grade to 55·0 per cent. for the O.F. grade, the 1 M. and the two inferior grades being in between. The percentages of the shoulder to the whole side do not show any significant differences between the grades. All the relative weights, except the initial weight, show an increase from the 1 L.S. grade to the O.F. grade with the 1 M. grade in between and the inferior grades corresponding more or less to the two first grades except in carcase weight where they are somewhat lower.

The small difference in length between the 1 L.S. and 1 M. grades are probably only due to the difference in weight, but the O.F. grade is shorter than the previous two. The 2 L.S. is the shortest of all the grades. The actual as well as the relative depth of the side increases from 1 L.S. grade to the O.F. grade. The ham also shows improvement. The evenness of the back fat improves with fattening and so does the thickness of the belly up to a point. The thickness of belly of the two inferior grades are much lower than the other grades. The marbling improves with fattening but is not affected by the inferiority of the two grades. When the depth at the flank is taken as 100 and the depth at the shoulder expressed as a percentage thereof, the inferior grades show a larger percentage than the other grades, the 1 M. grade being the lowest. The refractive indices correspond with the thickness of back fat of the different grades, the 1 L.S. grade having the softest fat and the O.F. grade the hardest fat.

The 268 males and 183 females are distributed among the five grades as shown below :—

	Males. Per cent.	Females. Per cent.
1 Lean Sizable	33·6	56·8
1 Medium	27·6	28·4
Overfat	12·7	4·9
2 Lean Sizable	20·9	9·8
2 Medium	5·2	—

The males only have 61 per cent. carcases in the two first grades whereas the females have 85 per cent. The 1 M.grade does not show much difference between the sexes, but the large difference is in the 1 L.S. grade which is also the most desirable grade of carcase. This large difference in favour of the females has also been found in the Scandinavian countries as shown by the percentages in table 13.

Table 13 has been prepared to show how some of the more important measurements of the 1 L.S. grade compare with the first grades of other bacon producing countries.

TABLE 13.—*Some Average Measurements of First Grade Carcases in Different Countries.*

Country.	Live weight.	Gain per day.	Length.	Depth.	Belly.	Back fat.	*Per cent. of the sexes.	
							M.	F.
	℔.	℔.	in.	in.	in.	in.		
Swedish (1)...........	201	1·09	29·56	—	1·311	1·44	28·3	71·7
Danish (2)...........	201	1·14	—	--	1·221	1·42	37·7	62·3
English (3)...........	200	·74	30·16	--	—	---	—	—
South African........	193	·99	28·94	15·87	1·2	1·411	37·4	62·6

(1) Hansson (1927). (2) Beck (1931). (3) Davidson and Duckham (1929).
*The percentages of the male and females are those when an equal number of both sexes are slaughtered without previous grading or selection.

The average live weight of the 1 L.S. pigs is below that of the first grade pigs of the other countries. This is, however, not of such importance as the average length of the 1 L.S. grade which is much below those of the two countries given. From general observation it appeared that a large percentage of the pigs sent to the bacon factories are deficient in length. The regulations of the new classes for bacon of recorded pigs, at the London Dairy Show, stipulate that a carcase of 140 to 149 lb. should have a length of 29·25 inches to qualify. The average carcase weight of the 1 L.S. grade is 144 lb. and the length only 28·94 inches. This is, therefore, the main point where improvement is necessary, since the other measurements agree well with those of the other countries.

3. *Effect of Weight and Sex on the Measurements.*

When the type of pig is the same, then weight plays an important part in determining the grade of the carcase in bacon production, and first grade carcases will fall in fairly narrow weight limits. To see what effect weight had on our results, the data have been grouped in three weight classes in which the sexes were kept separately. The actual and relative results are given in table 14.

There is no significant difference between the initial ages of the three weight groups and the sexes within the groups, while the final ages decrease with increase in weight, the females in all the groups being older than the males. For a better comparison the ages to reach

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a weight of 200 lb. for the different groups are also given. When the pigs weighed less than 200 lb., the gains made during the week before slaughter were used to determine the length of time they would have taken to reach the 200 lb. mark. The males took 7 days less to reach a live weight of 200 lb. than the females in the lightest group. The age of the males decreased about 10 days each time that the average live weight increased 11 to 12 lb., but the decrease in the case of the females is more rapid, so that in the heaviest group the ages to reach a weight of 200 lb. by the males and females, differs only by one day. Nearly the same changes are shown by the average daily gains of the pigs while in the feeding trials. This difference in gain between the light and heavy groups was also found by Hansson (1927). A factor that may have influenced this to some extent was, that the quickest growers had sometimes to be kept at the farms after they were ready for slaughter, before dispatching them to the factory so as to have large enough consignments. One would rather have expected that the lighter groups were those that had finished first, i.e. the quickest growers and not the opposite.

As regards the initial weights only the heaviest group is somewhat higher, indicating that the pigs had started to gain on the others at the commencement of the trials. The shrinkages en route, the carcase percentages, the percentage of cured sides and the weight of the shoulders do not show any significant differences between the different weight groups. In all the weight groups the females have lighter cured sides even where the average live weight was the same as for the males or slightly above, and the percentages of the cured sides show this difference. The difference in degree of fatness between the sexes may have affected this to some extent. Although only very small, the differences between the percentages of shoulder to whole side, are consistently smaller for the females than for the males in the separate groups.

The length increased ·3 to ·5 of an inch with an increase of 11 to 14 lb. in live weight. The correlation coefficient for farm live weight and length is $+0·48 \pm ·02$. Except for the lightest group, where the lengths are the same, the females are longer than the males.

The depth of the side increases with increase in weight, and has a correlation coefficient of $+0·5 \pm ·02$, which is about the same as that between weight and length. The ratio of shoulder to flank depth shows that, with increase in weight the flank depth increases more rapidly that the depth at the shoulder. In every weight group the males are deeper at the shoulder than the females, but the latter are again deeper at the flank and the average depth of side is also consistently larger for the females. This better development of flank to shoulder for the females is also clearly shown where the depth of the shoulder is expressed as a percentage of the depth at the flank. Wilkens (1929) found that the female develops strongly in all directions whereas the boar develops mostly in height and length during the first year, while breadth and depth development lags behind.

TABLE 14.—*Effect of Weight and Sex on Carcase Measurements.*

Weight Class.—lb.	Actual Measurements.						Relative Measurements.					
	189 and Below.		190–199.		200 and Above.		189 & Below.		190–199.		200 & Above.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
Sex.	M.	F.	M.	F.	M.	F.						
No. of pigs.	77	57	95	65	94	61						
Initial age—days.	72·7	70·1	71·0	69·9	71·3	71·7						
Final age—days.	189·3	194·6	188·3	190·0	184·8	187·8	164	163	160	155	176	177
Initial weight—lb.	46·6	46·2	46·3	45·0	51·5	52·4						
Final weight—lb.	183·7	183·0	194·6	194·6	206·8	209·4	647	644	673	669	708	707
Factory weight—lb.	168·7	168·1	178·4	179·6	190·0	194·4						
Per cent.	91·8	91·8	91·7	92·3	91·8	92·8						
Carcase weight—lb.	138·5	137·6	146·0	145·6	154·6	159·2	488	485	505	500	529	538
Per cent.	75·4	75·2	75·0	74·8	74·7	76·1						
Weight cured side—lb	49·0	47·9	51·1	50·3	54·6	54·3	173	169	177	173	187	183
Per cent. (2 sides)	53·3	52·3	52·5	51·7	52·8	51·9						
Weight of shoulder—lb	14·9	14·5	15·6	15·2	16·7	16·2	53	51	54	52	57	55
Per cent. of side.	30·4	30·3	30·5	30·2	30·6	29·8						
Average daily gain—lb.	1·234	1·145	1·298	1·299	1·444	1·4	100	100	100	100	100	100
Age at 200-lb. live weight—days.	200·0	207·1	190·4	193·8	181·0	182·1						
Length—inches.	28·4	28·4	28·9	29·1	29·23	29·6	100	100	100	100	100	100
Depth at shoulder—inches.	16·1	15·9	16·4	16·2	16·9	16·8	57	56	57	56	58	57
Depth at flank—inches.	15·2	15·6	15·6	15·8	16·1	16·5	54	55	54	54	55	56
Average—inches.	15·68	15·72	15·98	16·0	16·48	16·66	55	55	55	55	56	56
Ratio—per cent.	105·9	102·0	105·2	102·5	105·0	101·8						
Circumference ham—inches.	23·4	23·3	23·7	23·9	24·2	24·3	82	82	82	82	83	82
Average back fat—inches.	1·505	1·423	1·571	1·461	1·628	1·541	5·3	5·0	5·4	5·0	5·6	5·2
Evenness—per cent.	58·0	59·1	59·3	56·7	60·6	60·1						
Belly—per cent.	70·0	77·0	76·3	79·1	76·7	84·6						
Marbling—per cent.	73·3	65·0	70·4	66·8	73·6	67·4						
Refractive index.	1·4599	1·4598	1·4596	1·4597	1·4594	1·4596						
Classification. ILS.—per cent	40·3	63·2	33·7	66·2	25·5	41·0						
IM.—per cent.	14·3	12·3	34·7	26·1	31·9	46·0						
O.F.—per cent.	9·1	3·5	10·5	1·5	19·2	10·0						
Inf.—per cent.	36·4	11·0	21·1	6·2	23·4	3·0						

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Although the actual circumference of ham increases with an increase in weight, the relative measurement shows no difference between the groups. Within the weight limits studied, the hams have therefore made no improvement when the plumpness is determined as above.

The average thickness of the back fat increases with an increase in weight. The relative thickness increases as well. The correlation coefficient between farm live weight and thickness of back fat is $+ 0 \cdot 26 \pm \cdot 03$. In all the weight groups the females have thinner back fat than the males. The evenness of the back fat shows little difference between the groups. The thickness of the belly shows a definite increase and is higher for the females in the same weight group in spite of the fact that the females have the thinnest back fat. The marbling tends to remain the same, being better for the males in all the groups. Wilson and Morris (1932) made a study of the composition of rabbit carcases and found that the flesh of males and females differed markedly and that the greatest difference was in the fat content. The females had approximately 4 per cent. more fat than the males in the '' young '' group and approximately 6 per cent. more in the '' adult '' group. This seems to show the influence of the male sexual organs since the opposite happens in pigs where castrated males are compared with normal females. In humans the female also has more fat deposited than the male. Gramlich and Thalman (1930), working with steers, spayed heifers and open heifers, got results which are somewhat contradictory to those found with the pigs in so far that the open heifers were '' finished '' before the steers. It is possible that there may be differences with different animal species. At 5 months Hammond (1932) found that the ewe had a higher percentage of meat and a lower percentage of bone than either the wether or the ram and reckons that this may be associated with the ewe's more early maturity, for the actual growth made by the wether and ram at 5 months was greater. The wether showed greater development of fat than both the ram and the ewe and he says that the removal of either the ovary or the testes is stated to be accompanied by a deposition of fat in the body. This therefore agrees with our results where the barrows are fatter than the females.

Except for the weight of the shoulder and the thickness of the back fat, the relative measurements do not show any differences between the sexes. In all cases the relative measurements increase with an increase in weight.

The grading of the carcases in the three groups and the sexes in the different weight groups, is interesting. A better picture of the differences is given by diagram V. than the actual figures.

Between the lightest and second lightest weight groups there is hardly any difference in the percentages of 1 Lean Sizable carcases. The percentages of Overfat carcases are also the same. There is an increase in the percentage of 1 Medium carcases and a decrease in Inferior carcases. While the 180 to 189 lb. group has 65 per cent. first grade carcases, the 190 to 199 lb. group has $80 \cdot 4$ per cent. The heaviest weight group has $72 \cdot 2$ per cent. first grade carcases. There is, however, a decided decrease in the percentage of 1 Lean Sizable

DIAGRAM V.

Grading of Barrows and Gilts at Different Live Weights.
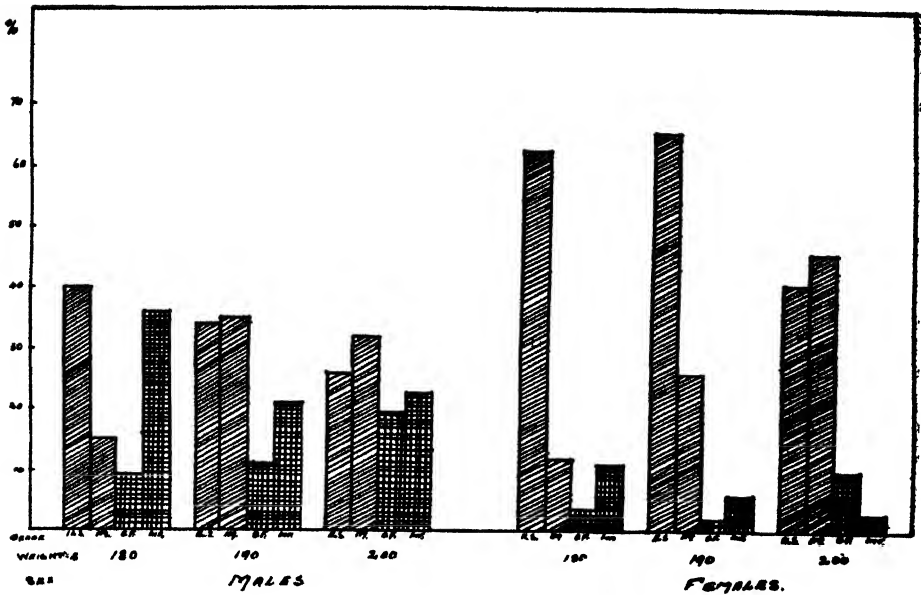
carcases and an increase in 1 Medium and Overfat carcases, while the percentage of Inferior carcases remains constant. From this it is clear that for bacon purposes the best weight at which the pigs should be slaughtered is between 190 and 199 lb. live weight on the farm. The carcase weight obtained is on the average 146 lb. This is borne out by the results obtained in Sweden and Denmark. In Denmark, for instance, the highest price is paid for first quality carcases when they weigh from 139 to 150 lb.

The males and females in the three groups show very marked differences as far as the grading is concerned. The males show a continual decrease in the percentage of 1 Lean Sizable carcases and an increase and then a decrease in 1 Medium carcases, with an increase in live weight. For first grade carcases (1 Lean Sizable and 1 Medium) the percentage rises from 54·6 per cent. to 68·4 per cent. and then decreases to 57·4 per cent. The Overfat carcases show a continual increase from 9·1 to 19·2 per cent. The percentage of Inferior carcases decreases. The females on the other hand first show a rise in the percentage of 1 Lean Sizable carcases and then a drop, while the 1 Medium carcases increase continuously. For first grade carcases the percentages from the lightest to the heaviest groups are 75·5, 92·3 and 87·0 per cent., respectively. There is an increase in Overfat carcases and a decrease in Inferior carcases. In the different weight groups the females have much higher percentages of first grade carcases than the males. The three main factors contributing to these differences in grading are, (a) the thinner back fat, (b) the thicker bellies, and (c) the slightly longer sides of the females. These very marked differences between the sexes have also been found in the Scandinavian countries where pig products are produced mainly

TABLE 15.—*Influence of Rate of Gain on Carcase Measurements.*

Measurement	200 & Above · 1·5 · F	200 & Above · 1·5 · M	200 & Above · 1 2-1·49 · F	200 & Above · 1 2-1·49 · M	200 & Above · 1·19 · F	200 & Above · 1·19 · M	190–199 · 1·5 · F	190–199 · 1·5 · M	190–199 · 1 2-1·49 · F	190–199 · 1 2-1·49 · M	190–199 · 1·19 · F	190–199 · 1·19 · M	189 & Below · 1·5 · F	189 & Below · 1·5 · M	189 & Below · 1 2-1·49 · F	189 & Below · 1 2-1·49 · M	189 & Below · 1·19 · F	189 & Below · 1·19 · M
No. of pigs	21	40	28	28	12	26	15	18	24	42	26	35	17	17	17	22	36	38
Initial age—days	75·4	74·0	68·6	70·0	72·2	73·9	71·5	68·7	69·5	69·4	69·4	74·2	67·6	67·6	67·6	72·5	71·8	75·2
Final age—days	170	162	186	187	223	218	161	160	178	180	218	213	151	151	167	175	213	215
Initial weight—lb.	57·8	57·9	50·3	49·9	46·2	43·5	50·8	48·6	46·0	45·5	40·7	46·1	50·6	48·3	46·8	46·8	40·0	44·7
Final weight—lb.	210	207	210	208	208	205	196	196	195	195	194	194	187	185	183	183	182	183
Factory weight—lb.	197	192	194	192	190	186	182	180	180	179	178	177	171	172	168	168	166	168
Per cent.	93·8	92·5	92·7	92·0	91·4	90·7	93·1	91·9	92·6	91·9	91·6	91·3	91·9	93·2	91·7	91·7	91·2	91·9
Carcase weight—lb.	164	156	159	156	155	152	147	147	148	147	144	144	138	141	139	139	137	138
Per cent.	77·8	75·4	75·6	74·8	74·7	74·2	75·0	75·1	75·9	75·5	74·0	74·3	74·6	76·2	75·8	75·8	75·2	75·7
Weight cured side—lb.	55·2	54·7	53·8	54·8	54·1	54·3	52·5	52·2	50·5	50·8	50·6	54·0	49·6	48·7	48·5	48·5	47·6	49·0
Per cent. (2 sides)	52·5	52·8	51·4	52·7	52·1	53·0	51·7	53·7	51·9	52·2	51·5	52·4	53·3	53·6	52·9	52·9	52·4	53·6
Weight shoulder—lb.	16·0	16·2	16·2	16·9	16·3	17·0	15·1	15·9	15·1	15·5	15·4	15·7	15·0	14·6	14·8	14·8	14·5	14·9
Per cent.	29·0	29·6	30·1	30·8	30·1	31·3	29·8	30·3	29·9	30·3	30·8	30·9	30·2	30·0	30·5	30·5	30·5	30·5
Average daily gain—lb.	1·63	1·72	1·37	1·36	1·07	1·10	1·62	1·61	1·37	1·35	1·05	1·07	1·63	1·37	1·33	1·33	·99	1·0
Age at 200-lb. wgt.—days	164	158	180	183	219	215	164	162	182	182	222	218	159	179	186	186	227	226
Length—inches	29·6	29·3	29·5	29·2	29·9	29·2	29·0	28·8	29·4	28·9	29·0	28·8	28·5	28·1	28·2	28·2	28·5	28·4
Depth shoulder—inches	17·0	16·9	16·7	16·0	16·7	16·7	16·4	16·5	16·2	16·3	16·1	16·4	16·2	15·8	16·0	16·0	15·9	16·1
Depth flank—inches	16·7	16·6	16·5	16·4	16·2	16·4	16·4	15·9	16·0	15·9	15·8	16·0	15·6	15·6	15·6	15·2	15·8	15·3
Average—inches	16·9	16·6	16·5	16·4	16·5	16·4	16·4	16·2	16·0	15·9	15·8	16·0	15·7	15·6	15·6	15·6	15·8	15·7
Ratio	102	104	101	105	103	105	101	104	103	105	103	106	106	106	105	105	102	105
Circumference ham—ins.	24·4	24·2	24·3	24·2	24·1	24·2	24·1	23·7	23·9	23·7	23·8	23·7	23·8	23·7	23·3	23·3	23·2	23·3
*Ratio.	82·4	82·6	82·4	82·9	80·6	82·9	83·2	81·3	81·3	82·0	82·1	82·3	83·5	83·0	82·6	82·6	81·4	82·0
Average back fat—inches	1·6	1·66	1·52	1·62	1·48	1·59	1·56	1·61	1·47	1·58	1·39	1·55	1·61	1·55	1·47	1·47	1·37	1·48
Evenness—per cent.	66·5	63·3	57·6	61·2	54·6	55·8	62·0	64·6	58·1	60·5	52·3	56·5	58·7	63·5	59·0	59·0	56·1	57·2
Belly—per cent.	86·2	77·5	83·9	78·6	83·3	73·5	84·0	78·3	78·8	77·4	76·5	74·0	68·8	78·8	69·1	69·1	75·6	70·8
Marbling—per cent.	67·5	74·4	66·8	71·5	68·9	68·5	72·0	77·1	65·7	67·0	64·5	70·8	72·4	68·2	70·5	70·5	64·1	76·3
Classification 1 L.S.—per cent.	19·1	15·0	50·0	28·6	58·3	38·5	46·7	27·8	58·3	28·6	84·6	42·9	72·4	41·2	40·9	40·9	72·2	44·7
Classification 1 M.—per cent.	52·4	35·0	46·4	42·9	33·3	15·4	46·7	33·3	37·5	45·2	3·9	22·9	8·3	23·5	18·2	18·2	8·3	13·2
Classification O.F.—per cent.	19·1	30·0	3·6	7·1	8·3	15·4	6·6	22·2	4·2	9·5	11·5	28·6	5·9	5·3	4·6	4·6	2·8	5·3
Classification Inf.—per cent.	9·5	20·0	—	21·4	—	30·8	6·6	16·7	—	16·7	—	5·7	29·4	35·3	36·4	36·4	16·7	36·8

* The circumference of the ham is expressed as a percentage of the length of the side.

in the form of bacon. Olson and Bull (1931) have analysed the data of ham and bacon belly yields of the carcases of 240 barrows and 205 gilts which were slaughtered at an average weight of 225 lb. They found that the average difference in dressing percentage was 0·5 per cent. in favour of the barrows, gilts averaged 0·39 per cent. more ham than the barrows; the bellies of the barrows were 0·23 per cent. heavier than those of gilts; loin yield was 0·43 per cent. in favour of gilts; barrows were 0·1 per cent. fatter, on an average, than gilts. With other comparisons they also found only very small differences and they come to the conclusion that from a commercial standpoint the differences are not significant. This is rather different to what our results and the Scandinavian results show. Whether the difference in slaughter weight and the difference in assessing the value, caused the difference in conclusions arrived at, is not clear. As far as the production of bacon is concerned, however, there can be no doubt that the gilts are superior to the barrows to a very marked degree.

4. *The Influence of the Rate of Gain.*

The idea has been commonly held that for bacon purposes pigs should not be allowed to grow too fast as they are then more liable to be too fat than when they are growing at a slower rate. In the reports of the East Anglian Pig Recording Scheme (1929) it came out clearly that the pigs in the herds making the slowest gains, gave the best results as far as grading is concerned. It is also well known that the slower the rate of gain the higher is the feed cost on account of the larger proportion of feed required for maintenance. Hansson (1927), however, on analysing the data of the Swedish testing stations concludes that, when pigs receive the proper feeding stuffs then the fast growers give better results than the slow growers as regards quality of carcases. In table 15 the average results are given after the data had been grouped according to the rate of average daily gains made by the pigs. The same groups have been used as in a previous part of the paper, and the live weights are kept constant. On account of the differences existing between the sexes, their results are given separately in the different weight and gain groups. This is also done in the subsequent groupings.

The initial ages show no consistent differences between the fast and slow growers, while the initial weights show an increase with increased rate of gain. At an age of about 10 weeks therefore, the quick growers already show a difference and this is more marked in the heavy than in the light groups.

The shrinkage on the journey shows a tendency to decrease with an increased gain. but this is rather a more indirect influence since the quick gainers are fatter and have deeper sides than the slow gainers. The same is the case with the dressing percentage and the percentage of cured sides. The small difference in the percentage of cured sides in favour of the males is again manifested. The proportion of shoulder to full side does not show much change, although the tendency is to decrease with that of the females slightly lower than that of the males.

TABLE 16.—*Effect of the Rate of Gain on the Length of the Side.*

Weight—lb.	189.			190-199.			189.			190-199.			200.		
Ave. depth of side—ins.	15-15·9			15-15·9			16-16·9			16-16·9			16-16·9		
Average daily gain—lb.	1·19	1·2– 1·49	1·5	1·19	1·2– 1·49	1·5	1·19	1·2– 1·49	1·5	1·19	1·2– 1·49	1·5	1·19	1·2– 1·49	1·5
No. of pigs..........	34	22	11	31	30	9	28	13	9	26	31	18	24	32	27
Length—inches........	28·44	28·24	28·59	28·73	29·25	29·09	28·4	27·89	28·01	29·21	28·99	28·81	29·45	29·43	29·04

In the 180-189 lb. group there is no significant difference between the length of the males and females, but in the other two weight groups the females are throughout longer than the males. There is, however, no consistent change in length with the increase in the rate of gain. The correlation coefficient for the rate of gain and the length of the side is + 0·13 ± ·03. The slight positive correlation coefficient is probably due to the increase in weight and not the increase in length since the weight was not kept constant in determining the correlation coefficient. The average depth of a side increases with an increase in the rate of gain. It appears that the increase in the depth at the flank is slightly more than at the shoulder. The depth of side and the rate of gain has a correlation coefficient of + 0·32 ± ·03 when the weight is not kept constant, so that weight may have influenced this also to some extent. The g·ouping of the data, however, shows that there is a definite correlation between depth of side and rate of gain. When the weight is kept constant then one would expect that with an increase in the depth of the side the length would rather decrease than increase. Since the depth and the rate of gain are quite strongly correlated there may be an indirect effect on the length when the rate of gain increases. Table 16 therefore represents the results of males and females where the weight and depth has been kept constant so as to see the effect of the rate of gain on the length.

Except in the last two depth groups, there is no consistent change in the length of the sides as the rate of gain increases. Only the last two groups show a consistent decrease in length. It therefore appears that rate of gain has only a very slight effect on the length of the side and that this slight effect is rather negative, i.e. length will rather decrease with an increase in rate of gain than the opposite.

There is a decided increase in the average thickness of the back fat with an increase in the rate of gain. The correlation coefficient is + 0·35 ± ·03. The evenness of the back fat increases as well, but there is no difference between the sexes. To eliminate any influence that may be due to the increase in the depth of the side as the rate increases, the weight and the depth have been kept constant in table 17 so as to see the effect of the rate of gain alone.

When only the rate of gain increases the thickness of the back fat still shows a definite increase.

In tables 14 and 15 it has been shown that the average thickness of the back fat of males and females increases as the weight and the average daily gain increase. To see whether the males or females fatten more quickly when the weight or rate of gain increases, the thickness of the back fat of the females have been expressed as percentages of that of the males. With an increase in weight the percentages are : —

Weight—lb.	180	190	200	210	220
Percentage..	94·6	93·0	94·6	90·6	94·2

TABLE 17.—*Effect of Rate of Gain on the Thickness of the Back Fat.*

Weight—lb.	· 189.			190–199.			189.			190–199.			200.		
Ave. depth of side—ins.	15–15 9			15–15·9			16–16·9			16–16·9			16–16·9		
Average daily gain—lb.	1·19	1·2–1·49	1·5	1·19	1·2–1·49	1·5	1 19	1·2–1·49	1·5	1 19	1·2–1·49	1·5	1·19	1·2–1·49	1·5
No. of pigs	34	22	12	30	30	10	28	13	9	26	31	18	24	32	28
Average thickness of back fat—inches	1·368	1·501	1·523	1·438	1·483	1·567	1 575	1·541	1·634	1 48	1 582	1·582	1·531	1·531	1·607

Weight apparently has no influence on the rate at which the back fat increases. The influence of the rate of gain seems to affect the males and females differently as shown below : —

Rate of Gain—℔......	1·19 and below	1·2–1·49	1·5 and above
Percentage...........	91·3	96·4	95·5

The same thing is shown in table 18 where the thickness of back fat at different rates of gain have been expressed as percentages of the back fat when the gain was the lowest.

TABLE 18.—*Influence of Rate of Gain on the Increase in Thickness of Back Fat of Males and Females.*

Average daily gain—℔.	·8–·9	1·0 1·1	1·2 1·3	1·4–1·5	1·6–1·7	1·8–1·9
Males.						
No. of Pigs.............	28	73	60	65	28	10
Thickness of back fat--in.	1·459	1·565	1·544	1·6	1·606	1·719
Relative thickness — per cent.....	100	107	106	110	110	118
Females.						
No. of pigs.............	30	44	41	48	17	3
Thickness of back fat--in.	1·36	1·423	1·462	1·581	1·518	1·67
Relative thickness — per cent	100	105	108	116	112	123

Both methods of expressing the rate of increase in the thickness of back fat indicate that as the rate of gain increases the thickness of the back fat of the females increases at a more rapid rate than that of the males.

The bellies show an improvement as the rate of gain increases but the marbling shows no consistent change but tend to decrease and then increase again. Since the thickness of back fat shows an increase as the rate of gain increases one would expect the marbling to improve. It may be that age had an influence since it is reckoned that marbling improves with age. In this case the quickest growers, and also the fattest pigs, were the youngest. In table 19 the thickness of back fat is kept constant to see the effect of rate of gain on marbling.

For both males and females the group with the thinnest back fat shows a continuous increase in marbling. In the other two groups, however, the slowest growers of both sexes have the best marbling, then there is a drop to the next gain group and then there is a slight rise again. To see the effect of age the data are grouped with the back fat again remaining constant while the age increases.

TABLE 19.—*Effect of Rate of Gain on Marbling.*

Thickness of back fat—inches	1·49 and Below.			1·5-1·74.			1·75 and Above.		
Average daily gain—lb	1·19	1·2-1·49	1·5	1·19	1·2-1·49	1·5	1·19	1·2-1·49	1·5
Males—									
No. of pigs	23	26	19	35	43	33	14	14	22
Marbling—per cent.	66·5	68·5	74·2	76·0	69·1	73·9	80	71·4	75·9
Females—									
No. of pigs	40	32	11	18	29	22	2	7	6
Marbling—per cent.	60·8	64·4	65·5	71·7	68·6	69·5	90	70	71·7

TABLE 20.—*Effect of Age on Marbling.*

Thickness of back fat—inches......	1·49 and Below.				1·5-1·74.				1·75 and Above.			
Age—days...................	140	160	180	200	140	160	180	200	140	160	180	200
Males—												
No. of pigs..............	15	14	18	18	21	30	27	36	16	14	6	14
Marbling—per cent........	75·3	70·0	66·1	66·7	76·2	68·3	67·4	76·1	78·8	72·1	76·7	77·9
Females—												
No. of pigs..............	12	19	21	31	11	12	27	19	—	—	—	—
Marbling—per cent........	66·7	63·6	60·5	62·3	70·9	70·1	67·4	71·6	—	—	—	—

This decrease and then again an increase (which is more marked than in table 19) in the marbling is also taking place when the age of the pigs increases, and this is very consistent in all the groups for males and females. We can give no explanation for this change unless there are two factors influencing the marbling; the one connected with early maturity, the effect of which decreases as the age increases, until the advanced age asserts its influence when the degree of marbling increases again.

Both males and females show rather marked decreases in the percentages of 1 Lean Sizable carcases as the rate of gain increases, while the 1 Medium carcases first increase and then decrease again. When the total percentage of first grade carcases are taken then there is first an increase from the slow growers to the medium fast growers and then a fall to the fast growers. From this it would therefore appear that there is an optimum rate of growth for baconers, viz., an average daily gain of 1·2 to 1·49 lb. This does not agree with Hansson's (1927) results where the quickest growers showed slightly better grading than the slow growers.

The results, as shown in table 15, show clearly that one cannot influence the length of the baconers to any marked extent by changing the rate of gain of the pigs. One can, however, influence the depth of the sides and the degree of fatness by changing the rate of growth. This is what one would expect knowing that length growth takes place early in the life of an individual whereas depth and width are later developing, especially in the males, as shown by Wilkens (1929). This is also the case with the amount of fat deposited. Bone and muscle development take place first and only then does a storage of fat take place [Hammond (1929)]. Length cannot therefore be influenced to a significant extent by the feed, and consequently we cannot agree with the conclusion arrived at by Schutte and Murray (1931), that barley had a favourable influence on the length of the pigs. It is only through breeding and selecting the required type of pig that one will succeed in altering the length. Slow growing pigs, being shallower than quick growing ones, may give one the impression that they are longer than the quick growers.

5. *The Influence of the Length of Side on the Carcase Measurements.*

Length in relation to the weight is one of the most important factors in judging the value of bacon sides. It is therefore important to know how the carcase measurements may be affected when we start increasing the length. For a definite weight, the ideal bacon pig should be long, deep and wide. It is, however, impossible to increase all these dimensions when the weight remains constant, consequently one or other measurement must suffer when length, for instance, is increased. The results where the carcases have been grouped according to length, are given in table 21.

TABLE 21.—Influence of Length on Carcase Measurements.

Weight—lb.	189 and Below						190–199						200 and Above					
Length—inches	28.4		28.5–29.4		29.5		28.4		28.5–29.4		29.5		28.4		28.5–29.4		29.5	
Sex	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
No. of pigs	42	24	27	27	8	5	20	10	52	29	22	26	11	4	35	19	47	37
Initial age—days	71·3	68·4	73·9	72·1	76·6	67·8	68·8	69·3	71·3	68·1	72·4	72·2	71·0	68·5	69·5	70·3	73·9	73·1
Final age—days	191	187	184	206	199	178	184	202	194	189	180	186	176	177	187	182	196	193
Initial weight—lb.	42·7	46·7	51·7	44·8	50	52·4	45·3	41·3	44·4	44·0	51·0	47·5	54·0	54·3	49·6	52·2	52·5	52·2
Final weight—lb.	183	184	185	183	186	185	194	195	194	194	196	195	204	203	206	207	208	212
Factory weight—lb.	168	169	169	168	172	168	179	180	178	179	178	180	188	189	189	193	191	196
Per cent.	92·0	92·2	91·4	91·7	92·3	90·8	91·9	92·5	91·7	92·3	91·2	92·3	92·1	93·0	91·9	93·4	91·8	92·5
Carcase weight—lb.	138	138	137	137	139	137	144	146	146	144	147	147	150	154	154	158	156	160
Per cent.	75·7	75·2	74·4	75·2	74·8	73·7	74·2	74·7	73·1	74·3	75·2	75·3	73·6	75·9	74·6	76·5	75·0	75·7
Wgt. cured side—lb.	48·9	48·0	49·3	47·6	48·3	50·0	52·1	51·3	51·0	49·3	50·6	50·8	53·9	—	54·4	54·4	54·9	54·3
Per cent (2 sides)	53·5	52·2	53·4	52·1	52·0	53·9	53·6	52·7	52·5	50·9	51·8	52·0	52·8	—	52·8	52·6	52·8	51·3
Wgt. shoulder—lb.	14·8	14·7	15·0	14·3	14·9	15·3	15·9	15·4	15·9	15·1	15·9	15·5	16·7	16·6	16·7	16·6	16·7	16·2
Per cent.	30·3	30·6	30·4	30·0	30·8	30·6	30·5	30·0	30·8	30·6	30·4	30·5	30·8	30·7	30·7	29·8	30·4	29·8
Ave. daily gain—lb.	1·22	1·21	1·28	1·06	1·18	1·3	1·32	1·23	1·25	1·29	1·37	1·33	1·52	1·43	1·39	1·44	1·47	1·37
Age at 200 lb. weight—days	202	198	195	219	208	187	186	205	198	194	184	189	173	175	183	179	181	185
Length—inches	27·8	27·7	28·8	28·8	30·0	29·6	27·9	28·0	28·9	28·9	29·8	29·9	27·8	28·0	28·8	29·0	29·9	30·0
Depth shoulder—in.	16·2	15·8	16·1	16·0	15·8	15·6	16·6	16·3	16·3	16·1	16·3	16·3	16·6	17·1	16·7	16·9	17·0	16·7
Depth flank—ins.	15·4	15·6	15·1	15·5	14·9	15·4	15·9	16·0	15·5	15·8	15·5	15·7	15·8	16·6	16·1	16·6	16·1	16·4
Average—inches	15·8	15·7	15·6	15·8	15·4	15·5	16·2	16·2	15·9	15·9	15·9	16·0	16·2	16·8	16·4	16·8	16·6	16·7
Ratio	105	101	107	103	106	101	105	102	105	102	105	104	105	103	104	102	105	102
Circ. ham—inches	23·4	23·4	23·3	23·4	23·4	23·2	23·9	23·8	23·6	23·9	23·8	24	24·8	24·2	24·1	24·2	24·2	24·4
Ratio	84·2	84·5	80·9	81·3	78·1	78·2	85·7	85·1	81·7	82·7	79·7	80·4	88·8	86·4	83·7	83·4	80·8	81·2
Ave. back fat—in.	1·55	1·48	1·47	1·39	1·37	1·34	1·66	1·54	1·57	1·44	1·49	1·46	1·55	1·56	1·63	1·55	1·65	1·54
Evenness—per cent	59·3	60·0	56·6	57·4	55·9	62·9	59·8	56·1	58·8	57·2	59·4	56·4	59·3	61·6	60·9	63·8	60·5	57·8
Belly—per cent.	71·9	76·3	67·0	78·1	70·0	78·0	75·0	84·0	77·1	80·0	76·8	76·2	79·1	87·5	76·3	83·1	76·2	84·9
Marbling—per cent.	74·3	65·7	70·5	65·9	78·0	65·0	70·0	58·0	70·7	67·3	70·0	67·2	68·2	72·5	72·3	66·1	76·0	67·4
Classification — L.S.—p.ct	31·0	54·2	40·7	70·4	88·9	60·0	10·0	70·0	38·5	58·6	45·5	73·1	45·5	50·0	22·9	31·6	21·3	43·2
M.—p. ct.	19·0	20·8	11·1	7·4	—	—	30·0	20·0	34·6	31·0	40·9	23·1	9·1	—	31·4	42·1	38·3	48·7
O.F.—p. ct.	11·9	8·3	7·4	—	—	—	25·0	—	3·4	3·4	4·5	—	27·3	—	17·1	21·0	19·1	2·7
Inf.—p. ct.	38·1	16·7	40·7	22·2	11·1	40·0	35·0	10·0	21·2	6·9	9·1	3·8	18·2	50·0	28·6	5·3	21·3	

The initial and final ages of the different length groups do not show any consistent tendency, neither is this the case when the ages to reach 200 lb. live weight are compared. The initial live weights vary and consequently no definite relation can be seen between length and average daily gain. As has been discussed above, length rather decreased with an increased rate of gain than the opposite. Hansson (1927), however, found that the long pigs made a larger average daily gain (0·638 Kg.) than the short pigs (0·611 Kg.). Hansson did not keep the weights constant in comparing the long and short pigs so that the former were on an average 2 Kg. heavier than the latter. In our results, and also found by Hansson, the heavy pigs made the quickest gains and it would, therefore, appear that the difference in live weight rather caused the difference in gains between the long and short groups, than the difference in length as was found by Hansson.

Although the tendency is not definite, it still appears that there is a slight decrease in dressing percentage and percentage of bacon sides with an increase in length. Hansson (1927) found a very small decrease in the dressing percentage of the long pigs and Larsson (1928) got a positive correlation between length of side and weight of head and feet. In table 21 the thickness of the back fat decreases as the length increased which was also the case with both Hansson's and Larsson's results. This would tend to influence the dressing percentage. When length increases one would, however, expect to find the head and legs somewhat heavier as was found by Larsson. In our results this will influence the percentage of cured sides, which was, however, very slight.

With an increase in length the depth of the side decreases when weight remains constant. The males in the heaviest group shows the opposite, but in the other groups the tendency seems to be definite. The correlation coefficient for length and depth of side is 0·11 ± ·05 when the live weight is 190-199 lb. The relative depth of the side decreases to a marked extent when the length increases. The circumference of the ham shows hardly any change, but when it is expressed as a percentage of the length then there is a decided decrease. If the length of the leg increases when the length of the side increases then the ham of a long pig will be much less plump that that of a short one, when the circumference is expressed as a percentage of the length of the side. This takes place when one looks at the different types of pigs. Hansson's (1927) results show a decrease in the points awarded for the hams when the length increased.

The average thickness of the back fat decreased with an increase in length. The males in the heaviest group show the opposite. The correlation coefficient for these two factors is – 0·03 ± ·03. The weight was not kept constant in determining the correlation coefficient and this resulted in no correlation since weight and thickness of back fat and weight and length are positively correlated. The effect of length is consequently neutralised when the weight increased with the length. Larsson (1928) found a negative correlation between length and the thickness of the back fat (– 0·28 ± ·07) when he kept the live weight constant. The length does not affect the evenness of the back fat significantly as shown in table 22 when the thickness of the back fat remains constant.

TABLE 22.—*Effect of Length on the Evenness of the Back Fat.*

Thickness of back fat—inches....	1·24 and Below.			1·25-1·49.			1·5-1·74.			1·75 and Above.		
Length—inches............	28·4	28·5	29·5	28·4	28·5	29·5	28·4	28·5	29·5	28·4	28·5	29·5
No. of pigs..............	3	14	6	38	67	55	57	82	57	15	25	27
Evenness—per cent..........	56·4	52·4	53·5	56·9	56·3	56·8	60·0	61·1	59·5	64·1	62·5	62·3

TABLE 23.—*Influence of Degree of Fatness on Carcase Measurements.*

Weight—lb. / Back fat—inches	189 and Below						190-199						200 and Above					
Back fat—inches	1·24	1·25-1·49	1·25-1·49	1·5-1·74	1·5-1·74	1·75	1·25-1·49	1·25-1·49	1·5-1·74	1·5-1·74	1·75	1·75	1·25-1·49	1·25-1·49	1·5-1·74	1·5-1·74	1·75	1·75
Sex	F.	M.	F.	M.	F.	M.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
No. of pigs	8	29	25	33	21	9	30	35	47	23	17	4	21	19	46	27	25	9
Initial age—days	75·8	74·4	69·8	72·0	68·9	67·4	72·3	70·9	71·9	71·5	66·2	60·3	72·1	71·1	72·3	71·4	70·7	71·2
Final age—days	206	191	194	188	191	183	192	193	188	185	182	170	188	193	188	185	176	181
Initial weight—lb.	50·8	50·2	47·8	44·5	42·3	39·3	46·7	48·3	46·9	42·5	42·4	38·0	49·6	53·4	51·7	49·9	51·7	53·4
Final weight—lb.	181	184	184	184	185	170	195	195	195	193	179	179	206	209	207	208	206	212
Factory weight—lb.	164	169	169	169	170	156	178	180	178	180	179	179	188	192	190	193	192	200
Per cent.	90·6	91·7	91·8	91·8	92·1	91·5	91·4	92·4	91·5	92·4	92·3	91·6	91·2	92·0	91·5	93·0	93·1	94·6
Carcase weight—lb.	130	139	137	139	142	141	145	145	146	148	147	148	154	156	154	158	155	167
Per cent.	72·1	75·6	74·5	75·5	76·8	75·7	74·6	74·3	74·8	75·7	76·0	76·1	74·8	74·9	74·5	76·2	75·2	78·6
Wgt. cured side—lb	47·4	47·5	47·1	49·3	49·5	51·6	49·3	49·9	51·5	50·6	54	52·7	54·2	52·7	54·2	53·5	56·8	58·6
Per cent (2 sides)	52·5	51·7	51·3	53·6	53·5	55·5	50·6	51·3	52·8	51·9	55·5	50·5	52·4	50·5	52·4	51·5	55·1	55·4
Wgt. shoulder—lb.	15·1	14·5	14·4	14·8	14·7	15·9	15·0	15·4	15·8	15·8	16·3	16·1	16·7	15·7	16·7	15·5	17·1	17·5
Per cent.	31·9	30·5	30·6	30·0	29·7	30·8	30·4	30·9	30·7	30·7	30·2	29·8	30·6	29·8	30·6	29·0	30·2	29·9
Ave. daily gain—lb.	1·02	1·19	1·14	1·27	1·21	1·3	1·27	1·23	1·31	1·38	1·34	1·48	1·39	1·31	1·41	1·45	1·55	1·48
Age at 200 lb. weight—days	219	202	207	199	202	192	195	197	191	189	186	173	186	188	184	181	173	173
Length—inches	28·8	28·5	28·4	28·3	28·3	27·9	29·2	29·2	28·7	29·1	28·8	29·2	29·1	29·6	29·1	29·5	29·4	29·3
Depth shoulder—in.	15·4	16·0	15·8	16·3	16·0	16·6	16·2	16·1	16·3	16·4	16·8	16·1	16·5	16·6	16·9	16·9	17·1	17·1
Depth flank—ins.	14·7	15·1	15·5	15·4	16·0	15·9	15·3	15·7	15·5	15·9	16·1	16·1	15·8	16·2	16·1	16·6	16·3	17·2
Average—inches	15·1	15·6	15·7	15·8	16·1	16·3	15·8	15·9	15·9	16·1	16·5	16·3	16·2	16·4	16·5	16·8	16·7	17·2
Ratio—per cent.	105	106	102	106	101	104	106	102	105	103	104	102	104	103	105	102	105	99
Circ. ham—inches.	23·4	23·3	23·3	23·5	23·4	23·3	23·6	24·0	23·8	23·8	23·6	24·1	24·1	24·0	24·2	24·3	24·3	24·6
Ratio—per cent.	81·3	81·9	82·0	83·0	82·6	83·6	80·8	82·2	82·7	81·7	82·6	82·5	82·7	81·2	83·1	82·4	82·7	83·2
Back fat—inches.	1·13	1·36	1·37	1·59	1·59	1·85	1·38	1·38	1·6	1·57	1·84	1·81	1·4	1·38	1·61	1·59	1·86	1·87
Evenness—per cent	49·7	56·9	57·5	58·4	64·3	62·1	56·4	54·8	60·1	59·3	62·8	64·5	59·1	57·7	59·8	62·9	63·7	59·4
Belly—per cent.	58·8	69·7	76·8	69·7	85·7	82·2	73·3	78·0	76·6	76·6	81·2	87·5	77·6	86·3	75·0	85·2	78·0	84·4
Marbling—per cent.	50·3	68·3	62·6	76·9	72·0	75·6	69·6	62·8	67·0	71·0	80·7	80·0	70·5	65·0	75·0	66·7	73·6	72·2
Classification — L.S.—p.ct.	37·5	69·0	92·0	24·2	38·1	11·1	50·0	85·7	34·0	47·8	47·1	25·0	37·1	84·2	26·1	22·2	40·0	44·4
M.—p.ct.	—	3·4	4·0	24·2	28·6	22·2	23·3	14·3	38·3	43·5	41·2	50·0	38·1	15·8	23·9	66·7	48·0	3·7
O.F.—p.ct.	—	—	—	3·0	4·8	66·7	26·7	—	6·4	4·3	11·8	—	—	—	10·9	3·7	12·0	7·4
Inf.—p.ct.	62·5	27·6	4·0	48·5	28·6	—	—	—	21·3	4·3	—	25·0	4·8	—	39·1	7·4	—	—

The thickness of the belly decreases with an increase in the length. The marbling shows no definite trend.

The percentages of 1 Lean Sizable carcases in the first two weight groups increase with an increase in length when the males and females are taken together. This is not so with the heaviest group where the shortest pigs have the highest percentage of 1 Lean Sizable sides. In all the groups there is a larger percentage of 1 Lean Sizable female carcases except in the longest group of the 180-189 lb. weight group where a large percentage (40 per cent.) of the female carcases were " unfinished ".

6. *The Influence of the Degree of Fatness on the Carcase Measurements.*

To see how fattening influences the carcase measurements the data have been grouped according to the thickness of the back fat. According to Larsson (1928) the thickness of the back fat is the best indication of the degree of fatness of a pig. The results are shown in table 23.

The initial and final ages decrease with an increase in the degree of fatness within the different weight groups. The initial weights also tend to decrease. The average daily gains increase as the thickness of the back fat increases and the correlation coefficient is $+ 0.35 \pm .03$. The ages to reach a live weight of 200 lb. decrease markedly, there being a difference of about 14 days between the fattest and leanest pigs in the three weight groups. The actual age and the thickness of the back fat is negatively correlated $(- 0.24 \pm .03)$. To see at what ages the rate of gain for the different fat groups show the largest gains, the average daily gains at different periods have been determined and expressed as percentages of the gains made from birth up to 90 days of age. Table 24 and diagram 6 show the trends of the different groups.

TABLE 24.—*Actual and Relative Gains made by Pigs of Different Degrees of Fatness.*

	No. of pigs.	Age in Weeks.						
		13	17	21	25	29	33	37
Males.								
Back fat, 1·24 and below—inches......	5	·824	1·15	1·394	1·398	—	—	—
Relative—per cent....		100	140	169	170	—	—	—
1·25-1·49—inch......	66	·702	1·122	1·4	1·589	1·573	—	—
Relative—per cent....		100	160	199	226	224	—	—
1·5-1·74—inches.....	124	·686	1·048	1·395	1·439	1·67	1·395	1·843
Relative—per cent....		100	153	203	210	243	203	269
1·75 and above—inch	68	·665	1·073	1·314	1·417	1·494	1·8	1·893
Relative—per cent....		100	161	198	213	225	271	285
Females.								
1·24 and below—inch	12	·667	·854	1·181	1·243	—	—	—
Relative—per cent....		100	128	177	186	—	—	—
1·25-1·49—inches....	74	·746	1·001	1·311	1·355	1·495	1·267	1·531
Relative—per cent....		100	134	176	187	200	170	205
1·5-1·74—inches.....	92	·654	1·029	1·231	1·32	1·325	1·501	2·276
Relative—per cent....		100	157	188	202	203	230	348
1·75 and above—inch	22	·663	1·037	1·269	1·293	1·158	1·479	2·028
Relative—per cent....		100	156	191	195	175	223	306

The proportion of males (263) and females (200) in the four groups are as follows:—

Thickness of back fat-inches	1·24	1·25—1·49	1·5—174	1·75
Males—per cent............	1·9	25·1	47·1	25·9
Females ., ,.	6·0	37·0	46·0	11·0

The females have a larger percentage of carcases falling in the classes with thin fat than the males. The two fattest male groups do not show much difference in relative gains, except for the irregularity at 8 months in the 1·5 – 1·74 inches group. The increase in gains are maintained up to 9 months although the steepness varies. The third group (1·25 – 1·49 inches) shows a drop from 6 months while the leanest group already remains constant from 5 months, and they also make lower relative gains than the other three groups. In the case of the females the two fattest groups are about the same and the two leanest ones slightly lower and also about the same. Up to about 2 months the difference in relative gains are not very pronounced. The males and females in the two leanest groups tend to remain constant or start decreasing in relative gains from 5 to 6 months, whereas those in the fatter groups continue to increase. The females show a depression from 5 to 7 months and then make more rapid gains than the males. Except for the male group 1·5—1·74 in., the males make more regular gains than the females.

DIAGRAM VI.
Relative Gains of Barrows and Gilts of Different Degrees of Fatness.
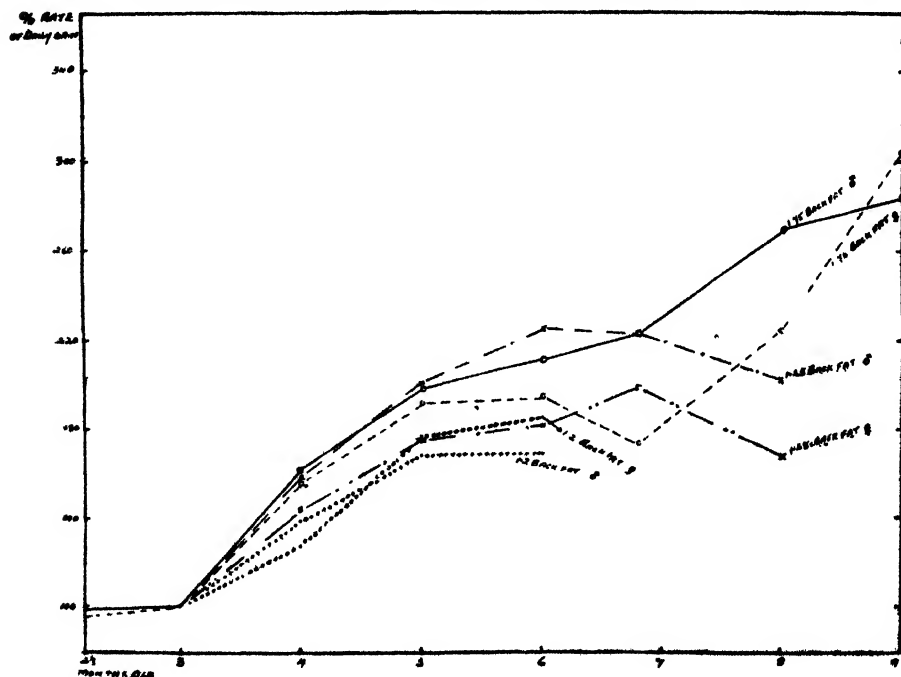
The shrinkage on the journey tends to decrease with increased fatness. The dressing percentage increased, so also the percentage of cured sides. The males do not show so consistently a higher percentage of cured sides over the females as in table 15 since the degree of fatness is the same in table 23. The proportion of shoulder to the whole side shows very little change but tends to decrease.

The length decreases with increased fatness. This has been fully discussed in a previous section. There is a marked increase in the depth of the side as the pig fattens. The flank measurement increased at a quicker rate than the shoulder measurement as shown by the shoulder/flank ratio. The females show quite a large difference from the males as regards this ratio and in the 200 lb. and heavier group the fattest females have a deeper flank than shoulder measurement. Not a single male carcase had a greater depth at the flank than at the shoulder. The correlation coefficient of depth of side and thickness of back fat is + 0·47 ± ·03. There is a slight increase in the circumference of the hams with an increase in fatness and when it is expressed as a percentage of the length of the side it shows a small improvement. The males and females do not show any consistent difference.

The evenness of the back fat improves as the thickness increases. The average measurements of the back fat expressed as percentages of the thickness at the shoulder for the three main grades are as follows : —

	Place Measured.			
	Shoulder.	Back.	Loin.	Ham.
1 Lean Sizable—per cent.........	100	68·2	57·6	64·7
1 Medium—per cent.............	100	69·9	62·6	76·5
Overfat—per cent..............	100	73·5	65·6	70·5

As the pigs fatten all the back fat measurements approach that of the shoulder measurement, i.e. the back fat becomes more even. When carcases are therefore judged one should first make sure that the thickness of the back fat is the same before taking evenness into consideration. The thickness of the back fat and its evenness has a correlation coefficient of + 0·31 ± ·03. The bellies show marked improvement as the pig fattens and the correlation coefficient is + 0·35 ± ·03. Davidson (1927), discussing the Swedish testing station results, concludes that the lesser evil is to have thin back fat and a thin belly rather than thick back fat and a thick belly. Although the thickness of the bellies are also considered in the Scandinavian countries, much more attention is paid to the thickness of the back fat in grading carcases. In England rather more attention is given to the bellies in laying down standards for bacon classes at shows. The marbling of the lean meat also improves with fattening, the males being the better in practically all the groups.

When the weight groups are not taken into consideration but only the degree of fatness of the pigs then the average percentages for marbling are as follows:—

Thickness of back fat—inches...	1·24	1·25–1·49	1·5–1·74	1·75
Males............................	66·0 (5)	70·0 (69)	72·5 (116)	76·0 (50)
Females.........................	56·0 (11)	64·0 (72)	69·7 (69)	73·3 (15)

In brackets the number of carcases are given from which the averages are obtained. The correlation coefficient for the thickness of the back fat and marbling is + 0·29 ± ·03. As shown above the marbling of the female carcases improve at a quicker rate, than those of the males, with fattening.

The very marked effect of the thickness of the back fat on the grading is shown by the decrease of first grade carcases as the thickness of the back fat increases. Below the percentages of carcases in the different grades are given when the weight and sex are not considered but only the thickness of the back fat. The actual numbers are given in brackets.

Thickness of back fat—inches..............	1·25–1·49	1·5–1·74	1·75
1 Lean Sizable—per cent....................	73·0 (116)	32·6 (61)	3·1 (2)
1 Medium—per cent........................	15·7 (25)	38·0 (71)	40·6 (26)
Overfat—per cent...........................	—	6·4 (12)	47·0 (30)
Inferior—per cent..........................	11·3 (18)	23·0 (43)	9·4 (6)

First grade carcases (1 Lean Sizable and 1 Medium) show a decrease from 88·7 per cent. in the leanest group to 70·6 per cent. in the second leanest and only 43·7 per cent. in the fattest group. The 1 Lean Sizable carcases show a very rapid decline when the back fat is thicker than 1·5 inches on an average. As was shown in table 12 the average thickness of 1 Lean Sizable carcases is 1·4 inches, the thickest average measurement being 2·0 inches and the thinnest 1·2 inches.

Larsson (1928) made a study of the influence of the degree of fatness on the amount of feed consumed per unit of gain. He also compared the Swedish results with the Danish results which agree well as shown in table 25.

TABLE 25.—*Influence of Fatness on Feed Requirements.*

Material from.	No. of Groups.	Live Weight of Group at Slaughter Kg.	Correlation between Back Fat and Feed Units per Kg. Gain.	Increase in Feed Units per Kg. Gain, when Thickness of Back Fat Increases 1 cm.
Sweden..............	178	89·0–95·9	+ 0·22 ± ·07	0·10
Denmark...........	861	89·0–95·9	+ 0·19 ± ·03	0·12
Denmark...........	181	90 –90·9	+ 0·20 ± ·07	0·12
Denmark...........	182	91·0–91·9	+ 0·21 ± ·07	0·15

The above is more or less what one would expect since a fattening animal requires more energy above the maintenance requirements than one that is still putting on flesh. No mention is made whether the factor of the rate of gain was taken into consideration. As our results show, the fat pigs are the quickest growers and it is a well known fact that quick growers need less feed per unit of gain than slow growers. If this factor had been taken into consideration the difference in feed requirements per unit of gain would have been less than the results obtained in the above table between pigs of different degrees of fatness.

7. *Influence of Depth of Side on Carcase Measurements.*

The results, after the data had been grouped according to the depth of the sides, are given in table 26.

As in the case with the degree of fatness (table 23) the initial and final ages decrease with an increase in the depth of the sides, while the initial weights are somewhat variable. The average daily gain increased and the correlation with depth of side is + 0·32 ± ·03. The loss of weight on the journey decreases as the depth of side increases and the dressing percentage shows quite a marked improvement and the improvement appears to be more pronounced than in table 23 where the degree of fatness increased. As the depth increased the thickness of the back fat also increased and may have influenced the shrinkage en route and the dressing percentages. To eliminate the influence of the degree of fatness on these factors, the depth is changed in the different fat classes as shown in table 27. In table 14 it was shown that the different weight groups had no significant influence on the loss of weight en route and the dressing percentage, so that weight is not taken into consideration in table 27. The data of the males and females have been taken together.

TABLE 26.—*Influence of Depth of Side on Carcase Measurements.*

Weight—lb.	189 and Below						190—199						200 and Above					
Depth—inches	14.9		15–15.9		16–16.9		15–15.9		16–16.9		17		15–15.9		16–16.9		17	
Sex	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
No. of pigs	12	4	30	37	34	16	43	28	44	31	8	6	19	8	57	27	16	24
Initial age—days	78.3	84.0	73.2	70.3	70.9	66.4	70.7	71.9	71.4	68.4	69.0	69.0	72.0	76.1	70.3	73.2	71.6	68.9
Final age—days	203	227	187	190	190	197	189	203	188	180	185	180	188	198	188	191	168	182
Initial weight—lb.	50.0	54.5	49.1	46.9	43.7	42.8	44.5	42.0	48.1	47.7	45.9	44.7	49.0	45.6	49.6	53.1	53.3	53.1
Final weight—lb.	182	179	183	182	186	185	195	194	194	195	195	195	206	203	206	209	211	212
Factory weight—lb.	166	162	168	168	171	171	177	178	179	180	182	183	187	181	189	194	196	199
Per cent.	91.2	90.5	91.9	91.9	91.9	92.2	90.7	91.8	92.3	92.5	93.5	93.8	90.8	89.2	91.9	92.8	92.9	94.1
Carcase weight—lb.	135	139	138	136	142	143	144	144	148	147	150	152	151	149	155	158	160	164
Per cent.	74.1	77.4	75.2	74.5	76.3	77.0	73.8	74.2	75.9	75.3	77.3	77.8	73.3	73.4	75.2	76.0	76.0	77.5
Wgt. cured side—lb	45.9	—	48.7	47.4	50.6	50.0	50.5	50.0	51.3	50.5	53.1	52.5	53.5	50.6	54.8	55.3	56.7	56.2
Per cent. (2 sides)	50.3	—	53.3	52.0	54.6	54.0	51.9	51.5	52.8	52.8	54.6	53.8	52.0	49.9	53.3	53.0	53.0	53.0
Wgt. shoulder—lb.	14.0	—	14.8	14.5	15.3	14.7	15.5	15.3	15.7	15.1	16.3	15.4	16.5	15.0	16.7	16.6	13.9	16.6
Per cent.	30.5	—	30.4	30.6	30.2	29.5	30.7	30.6	30.6	29.9	30.7	29.3	30.8	29.6	30.5	30	29.8	29.5
Ave. daily gain—lb.	1.1	.87	1.27	1.18	1.25	1.13	1.3	1.2	1.29	1.37	1.33	1.39	1.4	1.31	1.4	1.37	1.68	1.46
Age at 200 lb. weight—days	215	242	196	204	199	207	192	208	192	184	188	182	184	196	184	187	162	174
Length—inches	28.7	29.2	28.6	28.3	28.1	28.5	28.9	29.1	28.8	29.2	28.6	28.9	29.1	29.6	29.1	29.7	29.7	29.4
Depth shoulder—in.	15.2	14.6	15.9	15.7	16.7	16.7	15.8	15.6	16.7	16.5	17.4	17.4	16.0	15.8	16.8	16.6	18.0	17.4
Depth flank—ins.	14.3	14.0	15.0	15.3	15.9	16.5	15.0	15.4	15.9	16.4	16.9	16.9	15.2	15.2	16.1	16.2	17.1	17.2
Average—inches	14.7	14.3	15.4	15.5	16.3	16.6	15.4	15.4	16.3	16.3	17.1	17.1	15.6	15.5	16.5	16.4	17.5	17.3
Ratio—per cent.	106	104	107	102	105	101	106	103	105	102	103	103	105	104	104	103	105	101
Circ. ham—inches	23.3	22.9	23.4	23.4	23.6	23.4	23.8	24.0	23.7	23.9	23.4	23.8	24.0	23.8	24.1	24.2	24.6	24.5
Ratio—per cent.	81.2	78.4	81.9	82.8	83.5	82.2	82.4	82.2	82.3	81.8	81.8	82.4	82.6	80.4	82.9	81.6	82.9	83.5
Back fat—inches	1.33	1.16	1.48	1.4	1.59	1.54	1.55	1.42	1.59	1.48	1.73	1.59	1.6	1.35	1.61	1.49	1.72	1.64
Evenness—per cent	58.7	46.2	55.2	57.8	60.3	65.3	58.0	53.5	60.1	58.9	62.0	60.3	57.7	60.1	60.0	58.7	67.5	61.5
Belly—per cent.	60.0	57.5	66.7	76.0	77.1	84.4	72.1	71.8	79.3	81.3	82.5	86.7	73.2	77.5	77.0	85.6	80.0	85.4
Marbling—per cent.	62.9	25.0	70.0	62.7	79.0	71.9	66.5	71.8	79.3	69.7	83.8	81.7	68.4	87.5	72.9	68.8	82.7	69.6
Classification — L.S.—p.ct.	46.7	25.0														48.1	12.5	20.8
M.—p.ct.	6.7	—														48.1	31.3	50.0
O.F.—p.ct.	6.7	—														3.7	31.3	20.8
Inf.—p.ct.	40.0	75.0														—	25.0	8.3

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TABLE 27.—*Influence of Depth of Side on Shrinkage and Dressing Percentages.*

Thickness of back fat—in.	1·25–1·49.								
Depth of side—in......	15–15·9.			16–16·9.			17.		
	Live wgt.	Fact. wgt.	Carcase wgt.	Live wgt.	Fact. wgt.	Carcase wgt.	Live wgt.	Fact. wgt.	Carcase wgt.
No. of pigs............	88	88	88	60	60	60	7	7	7
Average weight—lb......	189	173	140	199	182	150	204	188	154
Per cent. of farm wgt...	—	91·3	74·1	—	91·8	75·3	---	92·0	75·1
Thickness of back fat—in.	**1·5–1·74.**								
Depth of side—in......	15–15·9.			16–16·9.			17.		
	Live wgt.	Fact. wgt.	Carcase wgt.	Live wgt.	Facr. wgt.	Carcase wgt.	Live wgt.	Fact wgt.	Carcase wgt.
No. of pigs............	68	68	68	81	81	81	20	20	20
Average weight—lb......	193	176	144	197	180	149	207	192	159
Per cent. of farm wgt...	--	91·0	74·4	—	91·7	75·6	—	92·8	76·7
Thickness of back fat- -in.	**1·75 and above.**								
Depth of side—in......	15–15·9.			16–16·9.			17.		
	Live wgt.	Fact. wgt.	Carcase wgt.	Live wgt.	Fact. wgt.	Carcase wgt.	Live wgt.	Fact. wgt.	Carcase wgt.
No. of pigs............	9	9	9	32	32	32	16	16	16
Average weight—lb......	195	177	144	198	184	151	203	190	157
Per cent. of farm wgt...	--	91·1	74·2	—	92·8	76·2	—	93·3	77·1

It is shown quite definitely that the depth of side has an influence on the shrinkage and dressing percentages quite apart from the fatness of the pigs. Hansson (1927), however, found that the deep pigs had a larger loss at slaughter than the shallow ones and he thought that the difference might be partly due to a larger stomach content of the deep pigs. His depth measurements were taken on the inside of the carcases and not on the outside where our measurements were taken. Whether this difference in taking the depth measurements of the sides caused these opposite results is not clear. The length shows a slight decrease with an increase in the depth. Hansson's deep pigs were longer than the shallow ones, but he did not keep the weight constant so that the former were 2·2 Kg. heavier than the latter and this may account to some extent for the difference of 1·6 cm. in length. The percentage of shoulder decreases slightly so also the ratio of the shoulder and flank measurement, the decrease in the shoulder/flank ratio appearing to be more marked in the females than in the males. The circumference of the ham increases and the ratio has a slight tendency to increase, but this is not quite consistent.

The thickness of the back fat increases as the depth of side increases the correlation coefficient being $+ 0·47 \pm ·03$. In table 15 it was shown that rate of gain had a strong influence on the thickness of the back fat so that the influence will exert itself here since rate of gain increases with an increase the depth of the side. In table 28 the effect of the depth of the side on the thickness of the back fat is shown when the rate of gain and the live weight are constant.

TABLE 28.—*Effect of Depth of Side on Thickness of Back Fat.*

Live weight—lb.	189.			190–199.			189.			190–199.			200.		
Average daily gain—lb.	1·19			1 19			1·2–1·49			1·2–1·49			1·2–1·49		
Depth of side—inches	14·9	15	16	15·9	16	17	14·9	15	16	15·9	16	17	15·9	16	17
Thickness of back fat—inches	1·27	1·37	1 58	1·44	1·48	1·8	1 33	1·5	1·54	1·49	1·58	1·59	1·54	1·58	1·56
No. of pig	12	34	23	31	26	4	4	22	13	29	31	5	11	32	13

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When the effects of weight and rate of gain are eliminated, as shown in Table 28, the thickness of the back fat still increases when the depth increases. Here again our results do not agree with Hansson's, who found that the deep pigs had less back fat than the shallow ones. The actual measurements of the back fat are not given but only the points awarded which were 13·3 for the deep carcases and 12·5 for the shallow carcases. It is only in one respect that our results agree with Hansson's in connection with the depth of the sides, and that is the average gains made by the pigs. He also found that the deep pigs made the quickest gains (633 gm. per day as compared with 601 gm. made by the shallow pigs) and required 180 days to reach a live weight of 90 Kg. while the shallow pigs took 185 days to reach the same weight. Table 29 shows that the depth of side has an increasing effect on the evenness of the back fat when the average thickness of the fat is constant.

The actual thickness of the fat over the shoulder decreases slightly and the thickness at the loin again increases slightly as the depth of the side increases.

On account of the influence of the thickness of the back fat on the grading of the carcases, the results as shown in table 26 cannot be taken as showing the effect of the depth of the sides. Table 30 has been prepared where the weight and the thickness of the back fat are kept constant so as to see whether depth of side had any marked effect on the grading.

From the results of table 30 one can see that the depth of side has a certain amount of influence on the grading. There appears to be an optimum depth round 15-16·9 inches and that the depth expressed as a ratio of the weight remains more or less constant, since the optimum actual depth shifts as the weight increases.

8. *Factors affecting the Firmness of the Back Fat.*

One of the most unsatisfactory conditions in bacon production is soft carcases since this condition causes a depreciation in the value of bacon to a very large extent. In a study made of this subject in the United States of America [Hankins and Ellis (1928)], it was indicated that the softness of the fat is responsible for the softness in the pig carcases and the products. Conversely, when the fat of a carcase is firm the carcase and the products are firm. Different methods of determining the firmness of the fat were used, and it was found that the refractive index of the fat was the best single method to use, and was consequently also used in determining the firmness of the fat of the pigs used in this analysis. Ellis and Isbell (1926) found that the refractive indices of the fats of the meat and the back fat are almost identical. Ranges from hard to oily fat caused no variation between the two. Leaf fat values on the other hand were considerably below those of the other samples, i.e. they were much firmer, and the difference between the leaf fat and the meat or back fat was not always a uniform one. Therefore, by taking samples of the back fat and determining the firmness thereof, one can see what the firmness of the carcase is like.

TABLE 29.—*Effect of Depth of Side on the Evenness of the Back Fat.*

Back fat—inches..	1·49 and Below.						1·5-1·74.						1·75 and Above.					
Depth—inches......	15-15·9		16-16·9		17-17·9		15-15·9		16-16·9		17-17·9		15-15·9		16-16·9		17-17·9	
Place measured*...	S.	L.	S.	L.	S.	L.	S.	L.	S.	L.	S.	L.	S.	L.	S.	L.	S.	L.
Males.																		
No. of pigs........	36	36	36	36	3	3	49	49	62	62	9	9	9	9	30	30	12	12
Average—inch.....	1·92	1·07	2·04	1·17	1·96	1·34	2·25	1·28	2·24	1·34	2·18	1·41	2·54	1·52	2·51	1·55	2·49	1·54
Evenness—per cent	55·4		57·0		68·2		56·8		59·7		64·6		59·6		61·7		62·0	
Females.																		
No. of pigs........	48	48	36	36	6	6	19	19	31	31	20	20	—	—	—	—	—	—
Average—inch.....	1·94	1·06	1·95	1·1	1·88	1·13	2·24	1·3	2·2	1·38	2·2	1·38	—	—	—	—	—	—
Evenness—per cent	54·7		56·3		60·1		58·0		62·5		62·5		—		—		—	

* S. Shoulder—thickest place.   L, Loin—thinnest place.

TABLE 30.—*Effect of Depth of Side on Grading.*

Weight—lb.	189.			190-199.			189.		190-199.			200.		
Back fat—inches	1·25-1·49			1·25-1·49			1·5-1·74		1·5-1·74			1·5-1·74		
Depth—inches	14	15	16	15	16	17	15	16	15	16	17	15	16	17
No. of pigs	9	33	15	33	26	4	24	28	29	38	4	15	33	23
1L.S.—per cent.	55·6	81·8	93·3	87·9	57·7	25·0	41·7	21·4	27·6	50·0	—	33·3	30·3	8·7
1M.—per cent.	—	6·1	—	6·1	26·9	50·0	12·5	35·7	37·9	39·5	75·0	13·3	39·4	60·9
O.F.—per cent.	—	—	—	—	—	—	—	7·1	—	7·9	25·0	—	12·1	8·7
Inf.—per cent.	44·4	12·1	6·7	6·1	15·4	25·0	45·8	35·7	34·5	2·6	—	53·3	18·2	21·7

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Hammond (1932) discussed the question of the firmness of the body fat and refers to the different investigators who have worked on this subject and showed how fat that is first deposited is the firmest. In the pig we therefore get that the leaf fat is the firmest, then the back fat below the streak, the outer back fat layer being the softest. The percentage of unsaturated fatty acids causes the fat to be firm or soft. Bhattacharya and Hilditch (1931) found that the variation in the amount of unsaturated acids was mainly compensated by corresponding changes in the stearic acid content. The proportion of linoleic acid (1) clearly increased with an increase of unsaturation in the fatty oil forming part of the diet and (2) also increased from the leaf fat to the outer layer of the back fat. They further showed that when different rations were fed this order was still maintained and that an alteration in the diet had relatively less effect on the composition of the outer layer of back fat than on that of the inner layer or of the leaf fat. The outer layer of fat was the most unsaturated and contained a higher proportion of linoleic to oleic acid than the inner layer of back fat which was approximately more nearly in composition to the leaf fat than to the outer back fat, but being less saturated than leaf fat. They reckoned that the relative constancy of the outer back fat in composition may be determined by the adjustment of the fat nearest the skin to a more or less constant consistency adapted to the average temperature conditions of the external atmosphere. Moulton (1929) has suggested that the temperature at which fat is deposited may affect the composition of the fat, and then mentions the difference in melting point of the fat of seals and bears living in Arctic regions, which is softer than the fat of animals living in temperate climates. Animals having different body temperatures show differences in the firmness of their fats. The sheep with a body temperature of 104° F. has a firmer fat than the pig or dog with a body temperature of 101° F.

In the United States extensive co-operative investigations have been done on the influence of different feeds on the firmness of the fat and reports were issued by the Bureau of Animal Industry (1926, 1928). In Canada experiments have also been conducted in connection with the causes of soft bacon and the results were summarised by Day (1922) : —

(i) Lack of maturity. Generally speaking, the more immature the pig, the greater is the tendency to soft fat. Almost invariably the largest percentage of softness occurs among the light sides of bacon.

(ii) Lack of finish. Thin animals have a marked tendency to produce soft bacon. Marketing pigs before they are finished is, no doubt, responsible for a great deal of softness.

(iii) Unthriftiness, no matter what the cause may be, at most invariably produces soft bacon.

(iv) Lack of exercise has a tendency to produce softness, but this tendency can be largely overcome by judicious feeding.

(v) Exclusive grain feeding is, perhaps, one of the most common causes of softness.

(vi) Maize. Of the grains in common use, maize has the greatest tendency to produce softness.

(vii) Beans seem to have a more marked effect than maize in producing softness.

Breed differences in firmness of the fat have been determined at the Purdue University (Kelly, 1932). The fat of pigs of the Berkshire, Large White, Tamworth, and other British breeds became hard at about 80 to 100 lb. live weights while the American breeds—Poland China and others—particularly when of the "large type", were soft, in some cases up to 250 lb. live weight.

The effects of the different feeds on the hardness of the fat of the carcases dealt with in this paper, have been reported on elsewhere [Romyn and others (1930), Schutte and Murray (1931)], so that in the present paper the effect of feed will not be considered but the other factors which may influence the firmness of the fat as determined by the refractive indices.

Except in the case of a limited number of carcases, the outer and inner layers of the back fat samples were not rendered separately. In the few cases that this was done separately, the outer layer was softer than the inner layer as shown by the average refractive indices of 13 samples. When the two layers were not separated, the refractive index was 1·4597, the outer layer alone 1·4601 and the inner layer alone 1·4596. All the refractive indices values are at a temperature of 40° C.

In this paper the data have been grouped so as to see the influence of (1) the degree of fatness, (2) the rate of gain, (3) live weight, and (4) age. The correlation coefficients of these different factors and the refractive indices have been determined and are given below :—

(1) Thickness of back fat and refractive index.. — 0·48 ± ·03
(2) Rate of gain and refractive index............ — 0·30 ± ·03
(3) Live weight and refractive index............ — 0·23 ± ·03
(4) Age and refractive index.................... + 0·19 ± ·02
(5) Thickness of back fat and rate of gain...... + 0·35 ± ·03
(6) Thickness of back fat and live weight....... + 0·26 ± ·03
(7) Thickness of back fat and age.............. — 0·24 ± ·03
(8) Rate of gain and live weight.............. + 0·32 ± ·03
(9) Live weight and age...................... — 0·09 ± ·03

In determining the correlation coefficient of two factors no other factor was kept constant. They, however, give a good indication of the many factors which may influence the firmness of the fat and also how one factor may indirectly influence the firmness by influencing another factor.

(1) The thickness of the fat shows the strongest correlation with the firmness of the back fat and by grouping we get the following averages : —

Thickness of back fat—inches...	1·24	1·25–1·49	1·5–1·74	1·75
No. of pigs....................	12	119	151	52
Average refractive index........	1·4602	1·4598	1·4595	1·4593

There is a progressive decrease in the refractive index of the back fat with increased thickness, i.e., there is a progressive hardening of the fat. On account of this strong influence and also the correlation of the thickness of back fat with the other factors, the thickness of the back fat will be kept constant in determining the effect of the other factors on the firmness of the fat.

(2) Rate of gain.—In table 31 the influence of the rate of gain on the refractive index is shown.

The results in table 31 show quite clearly that when the thickness of the back fat is kept constant then the rate of gain has no influence on the firmness of the fat. Helmreich (1929) reckoned that when other conditions are equal then the quick growers have the firmest fat, but he did not say whether the thickness of the back fat was one of the conditions that remained constant. The correlation coefficient of the rate of gain and the refractive index (– $0·30 \pm ·03$) was only caused by the positive correlation (+ $0·35 \pm ·03$) between the rate of gain and the thickness of the back fat.

(3) Live weight.—Table 32 shows the influence of farm live weight on the firmness of the fat. ·

Although the average refractive indices do not show quite the same consistency as in table 31, one can nevertheless conclude that the live weight has no effect on the firmness of the fat when the thickness of the back fat is kept constant. The same has happened with the correlation shown between the live weight and the refractive index (– $0·23 \pm ·03$) as the rate of gain and refractive index. The live weight is also positively correlated (+ $0·26 \pm ·03$) with the thickness of the back fat and hence the correlation it shows with the firmness of the fat.

(4) Age.—This is the only factor of the four which shows a positive correlation with the refractive index, i.e. the older pigs have the softer fat. Table 33 shows its influence when the thickness of back fat is constant.

TABLE 31.—*Influence of Rate of Gain on Firmness of the Fat.*

Thickness of back fat—inches	1·24 and Below.			1·25-1·49.			1·5-1·74.			1·75 and Above.		
Average daily gain—lb	1·19	1·2	1·5	1·19	1·2	1·5	1·19	1·2	1·5	1·19	1·2	1·5
No. of pigs	12	2	—	72	31	17	57	49	36	20	15	24
Refractive index	1·4603	1·4604	—	1·4599	1·4599	1·4594	1·4595	1·4595	1·4595	1·4593	1·4593	1·4594

TABLE 32.—*Influence of Live Weight on the Firmness of the Fat.*

Back fat—inches	1·24 and Below.			1·25-1·49.			1·5-1·74.			1·75 and Above.		
Live weight—lb	180	190	200	180	190	200	180	190	200	180	190	200
No. of pigs	10	6	—	49	43	22	52	52	49	9	16	26
Refractive index	1·4603	1·4601	—	1·4598	1·4598	1·4596	1·4596	1·4595	1·4595	1·4592	1·4594	1·4594

TABLE 33.—*Influence of Age on the Firmness of the Fat.*

Back fat—inches	1·24 and Below.			1·25-1·49.				1·5-1·74.				1·75 and Above.		
Age—days	160	180	200	140	160	180	200	140	160	180	200	160	180	200
No. of pigs	3	9	11	22	22	48	60	12	25	48	56	13	6	21
Refractive index	1·4605	1·4601	1·4596	1·4601	1·4595	1·4595	1·4598	1·4593	1·4597	1·4595	1·4595	1·4592	1·4593	1·4594

In the first two groups the average refractive indices are somewhat variable but remain constant in the last two. From these results it therefore appears that age has no effect on the firmness of the back fat when the thickness of the latter is kept constant. There is a negative correlation between the age of the pigs and the thickness of the back fat (– 0·24 ± ·03) which resulted in a small positive correlation (+ 0·19 ± ·02) between the age and the refractive index.

From the evidence as given and discussed, one cannot come to another conclusion than that apart from such factors as feed, breed and climate, the thickness of the back fat, or the degree of fatness of the pig, is the only factor which affects the firmness of the back fat. The different factors influencing the firmness of carcases as given by Day (1922), such as lack of maturity, lack of finish and unthriftiness, are true in so far as these conditions affect the thickness of the back fat and so indirectly affect the firmness of the fat. Directly these factors have no effect on the firmness of the fat. The fact has been mentioned above that the outer layer of the back fat is much less affected by feed than the inner layer and if it is true that the composition of the outer layer remains practically constant under the same environmental conditions, then one would not expect that it would either change to an appreciable extent as the back fat thickens. The firming up will then be more due to the change taking place in the inner layer and the outer layer making out less and less of the total fat on the back. The average refractive indices given of the outer and inner layers separately and the two together, show that the average of the back fat (1·4597) is much nearer to that of the inner layer (1·4596) than to that of the outer layer (1·4601). These results also indicate on which lines some future investigations on the firmness of the fat could be carried out.

IV. SUMMARY.

The paper comprises an analysis of growth and carcase measurements of 450 to 550 baconers of the Large White × Large Black (sow) and Tamworth × Large Black (sow) crosses.

Growth.

1. *Preweaning.*—The average gestation period for 39 farrowings was 113·7 days, the average birth weight of the pigs being 2·89 lb. The males averaged 2·99 lb. and the females 2·78 lb. at birth and 8·6 per cent. of the males and 8·3 per cent. of the females were born dead. The average weight per pig decreased from 3·5 lb. and 3·17 lb. for males and females respectively, when the litter size was 6 to 8 pigs per litter, to 2·34 lb. and 2·21 lb. respectively, when the litter size was 15 to 17 pigs per litter. The data indicate that litters of more than 12 pigs per litter are not desirable. 81 per cent. males and 76 per cent. females of those born alive respectively, were weaned, and the difference is probably due to the difference in live weight between the sexes.

2. *Weights at Different Ages.*—The average live weight at 8 weeks (weaning) is 30 lb. The maximum variability of weight appears to be between 4 and 5 months. The barrows are more

variable than the gilts. Fast and medium fast growing males are also higher than the females but the slow growers show no difference in variability between the sexes. The correlation coefficients between weights at 4 and 8 weeks are $+ 0.76 \pm .02$ and 13 and 21 weeks $+ 0.89 \pm .01$.

3. *Rate of Gain.*—The relative weights of males decrease from birth and from 9 to 13 weeks are below the females and then show a definite rise and they outstrip the females from round 4 months. The relative gains are given for fast, medium-fast and slow growers of the two sexes, females being lower throughout. The differences increase and afterwards decrease again.

Factory Results.

1. The average results of the two crosses do not show marked differences; there are, however, large differences between the average results of the progeny of different boars of the same breed.

2. *Average Measurements of the Grades.*—There are 24 per cent. more females in the best grade than males. The best grade, 1 Lean Sizable, is deficient in length when compared with the requirements of the English market.

3. *Influence of Weight and Sex.*—The heaviest pigs made the quickest gains and have longer and deeper sides, and the correlation coefficients between weight and length and weight and depth are respectively $+ 0.48 \pm .02$ and $+ 0.5 \pm .02$. Barrows are deeper than gilts at the shoulder but the latter are deeper at the flank and also have a larger average. The barrows are fatter and have better marbling than the gilts, but the latter nevertheless have better bellies. The most suitable live weight at which to kill baconers lies between 190 and 199 lb.

4. *Influence of Rate of Gain.*—Depth of side increases with rate of gain, the correlation being $+ 0.32 \pm .03$. It has hardly any effect on length of side. Back fat increases with increase in rate of gain, the correlation being $+ 0.35 \pm .03$. The females have thinner back fat than the males, but fatten at a more rapid rate with increased gain and so tend to approach that of the males. Bellies improve and marbling decreases and increases again. This also takes place when age increases. For baconers the optimum gain per day appears to be between 1.2 to 1.49 lb. The results show that length cannot be significantly influenced by rate of gain since it is early maturing. Later maturing parts as depth and thickness of back fat can be influenced significantly.

5. *Influence of Length.*—The actual depth of side appears to decrease slightly when length increases whereas the relative depth decreases markedly. The thickness of back fat decreases.

6. *Influence of Degree of Fatness.*—The relative gains made by pigs of different degrees of fatness are shown. The shrinkage en route decreases and the dressing percentage and percentage of cured sides increase with increased fatness. Length decreases and there is a marked increase in the depth of the side (correlation $+ 0.47 \pm .03$), the flank measurements increasing at a quicker rate than the shoulder

measurements. Females have a smaller proportion of shoulder than males. Hams show a slight improvement. Evenness of back fat improves (correlation + 0·31 ± ·03) and also the bellies (correlation + 0·35 ± ·03), and the marbling (correlation + 0·29 ± ·03), the males having better marbling than the females.

7. *Influence of Depth of Side.*—The shrinkage en route decreases and the dressing percentage increases more markedly with increase in depth than with increase in fatness and it is still the case when thickness of back fat is kept constant. The proportion of shoulder decreases so also the relative depth at the shoulder. The thickness of back fat still increases with depth of side when gain is constant, and the evenness of the back fat increases with depth when thickness remains constant. Depth of side appears to have an influence on the grading, the optimum being 15 to 16·9 inches, and that depth/weight ratio remains about the same.

8. *Factors affecting the Firmness of the Fat.*—The refractive indices were determined to get a measure of the firmness of the fat. The correlation between refractive index and thickness of back fat is − 0·48 ± ·03, rate of gain − 0·3 ± ·03, live weight − 0·23 ± ·03 and age + 0·19 ± ·02. There are also correlations between these different factors and thickness of back fat. When the thickness of back fat is kept constant then live weight and the rate of gain have no influence on the firmness of the fat and the data also indicate that age has no influence either. These factors only affect the firmness of the fat, since they are correlated with the thickness of the back fat.

V. ACKNOWLEDGMENTS.

VI. LITERATURE.

ACKERSON, C. W., AND MUSSEHL, F. E. (1930). Sex Differences in the Normal Growth Rate of Chicks. *Jnl. Agric. Res.*, Vol. 40, No. 9, pp. 863-866.

BAETHGE, G. (1932). Mästungs und Ausschlachtungsversuche an Schweinen. *Tierernährung*, Vo. 4, No. 4, pp. 270-322.

BECK, N. (1931). 19de Beretning om sammehligende Forsög med Svin fra statsanerkendte Avlscentre. Issued by the Kgl. Veterinaer-og Landbohöjskoles, Copenhagen.

BHATTACHARYA, R., AND HILDITCH, T. P. (1931). The Body Fats of the Pig. 1. Influence of Ingested Fat on the Component Fatty Acids. *Biochem. Jnl.*, Vol. 25, Part 2, pp. 1954-1964.

BLOCK, H. W. (1930). Effect of Method of Rendering on the Refractive Index of Fats. *Jnl. Assn. Offic. Agric. Chem.*, Vol. 13, No. 1, p. 142.

BORISS, U. (1927). Zur Kenntnis des Nährstoffbedarfs für die Saügings- und Anschliessende Schnellmastperiode nebst biometrischen Messungsergebnissen beim deutschen veredelten Landschwein. *Zeitsch. f. Tierzüchtung u. Zuchtungsbiologie*, Vol. 10, pp. 69-144.

BUCHANAN SMITH, A. D. (1930). Litter Size. Is it Inherited? *Pig Breeders' Annual*, Vol. 10, pp. 46-52.

CALDER, A. (1930). Commercial Pig Production in Scotland. *Trans. Higland and Agric. Soc. Scot.*, Vol., 42, pp. 118-127.

CALDER, A., AND BUCHANAN SMITH, A. D. (1928). Pig Testing: The Results of Preliminary Work on Bacon Type. *Scot. Jnl. Agric.*, Vol. 11, No. 3, pp. 318-325.

CALMA, P. C. (1926). A Study of the Rate of Growth of Berkshire Native Pigs under Ordinary Conditions. *The Philippine Agriculturist*, Vol. 15, No. 6.

CARMICHAEL, W. J., AND RICE, J. B. (1920). Variations in farrow, with Special Reference to the Birth Weight of Pigs. *Univ. Ill. Agric. Exp. Stn., Bull.* 226, pp. 1-95.

CRAFT, W. A. (1929). The Influence of Birth Weight upon Subsequent Development of Inbred and Outbred Pigs. *Am. Soc. Animal Production*, Ann. Meeting 1929, pp. 128-130.

CULBERTSON, C. C., AND OTHERS (1931). Swine performance record—litter comparisons. *Iowa Sta. Bull.* 277, pp. 85-116. Abstr. in *Exp. Stn. Record* (1931), Vol. 65, No. 1, p. 63.

DAVIDSON, H. R. (1931). The most profitable weight for marketing pigs. *Scot. Jnl. Agric.*, Vol. 14, No. 1.

DAVIDSON, H. R. (1929). Pig records and production indices. Paper read at the Ann. Conf. Agric. Education Assn., July, 1929.

DAVIDSON, H. R. (1927). Variation in carcase type for pork and bacon. *Scot. Jnl. Agric.*, Vol. 10, No. 4.

DAVIDSON, H. R. (1926). Pig improvement. I. Commercial requirements. *Scot. Jnl. Agric.*, Vol. 9, No. 2, pp. 146-156.

DAVIDSON, H. R. (1926). Pig improvement. II. Meeting commercial requirements. *Scot. Jnl. Agric.*, Vol. 9, No. 3, pp. 262-271.

DAVIDSON, H. R. (1930). Costs, standards and results obtained in commercial pig keeping. Unpublished m.s.

DAVIDSON, H. R., AND DUCKHAM, A. N. (1929). East Angilan Pig Recording Scheme. First report, June, 1929, pp. 1-48.

DAVIDSON, H. R. AND BUCHANAN SMITH, A. D. (1927). Pig testing and recording for the purposes of "Advanced Registry". *Pig Breeders' Annual*, 1927-28, p. 48.

DAY, G. E. (1922). Productive swine husbandry. J. B. Lippincott & Co., Philadelphia, Penn., U.S.A.

DECHAMBRE, P. (1929). Einfluss des Kastration bei Schweinen in verschiedenen Alter. *Revue de Zootechnie*, Febr., 1929, p. 121. Abstr. in *Zuchtungskunde* (1930), Vol. 5, No. 2, p. 90.

DUCKHAM, A. N. (1929). East Anglian Pig Recording Scheme. Second Report, Oct., 1929, pp. 1-17.

EATON, O. N. (1932). Correlation of hereditary and other factors affecting growth in guinea pigs. *U.S. Dept. Agr., Tech. Bull.* 279, pp. 1-35.

EDITORIAL (1932). Value of show competitions. *New Zealand Dairyman*, Vol. 36, No. 10, p. 83.

ELLIS, HAVELOCK (1930). Man and Woman. 6th Ed. A. & C. Black, Ltd., London.

ELLIS, N. R., AND HANKINS, O. G. (1925). Soft pork studies. I. Formation of fat in the pig on a ration moderately low in fat. *Jnl. Biol. Chem.*, Vol. 66, No. 1, pp. 101-122.

ELLIS, N. R., AND ISBELL, H. S. (1926). Soft pork studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. *Jnl. Biol. Chem.*, Vol. 69, No. 1, pp. 219-238.

ELLIS, N. R., AND ISBELL, H. S. (1926). Soft pork studies. III. The effect of food fat upon body fat as shown by the separation of the individual fatty acids of the body fat. *Jnl. Biol. Chem.*, Vol. 69, No. 1, pp. 239-248.

ELLIS, N. R., AND ZELLER, J. H. (1930). Soft pork studies. IV. The influence of a ration low in fat upon the composition of the body fat of hogs. *Jnl. Biol. Chem.*, Vol. 89, No. 1, pp. 185-197.

GRAMLICH. H. J., AND THALMAN, R. R. (1930). Sex and age as factors in cattle feeding. *Nebr. Sta. Bull.* 252. Abstr. in *Expt. Station Record* (1931), Vol. 64, No. 7, p. 657.

HAINES, G. (1931). A statistical study of the relation between various expressions of fertility and vigour in the guinea pig. *Jnl. Agric. Res.* (U.S.A.), Vol. 42, No. 3, pp. 123-164.

HAMMOND, J. (1932). Growth and development of mutton qualities in the sheep. Oliver and Boyd, Edinburgh.

HAMMOND, J. (1922). On the relative growth and development of various breeds and crosses of pigs. *Jnl. Agric. Sc.* (Eng.), Vol. 12, Part 4, pp. 387-423.

HAMMOND, J. (1925). Reproduction in the rabbit. Oliver and Boyd, Edinburgh.

HAMMOND, J. (1929). Probleme der Fleischerzeugung. *Züchtungskunde*, Vol. 4, No. 12, pp. 543-562.

HANKINS, O. G., AND ELLIS, N. R. (1926). I. Some results of soft pork investigations. *U.S. Dept. Agric.*, Bull. 1407.

HANKINS, O. G., AND ELLIS, N. R. (1928). II. Some results of soft pork investigations. *U.S. Dept. Agric.*, Bull. 1492.

HANSSON, NILS (1927). Leistungskontrolle in der schwedische Schweinezucht. *Zeitschr. f. Tierzüch. u. Zuchtungsbiologie*, Vol. 10, No. 3, pp. 341-376.

HAZELTON, J. M. (1930). National swine record of performance. *Poland China Journal*, No. 17, No. 2. Abstr. in *Züchtungskunde* (1931), Vol. 6, No. 4, p. 157.

HEIDENREICH, C. H. (1930). Qualitätsbeeinflussende Faktoren beim Fleisch. *Zuchtungskunde*, Vol. 5, No. 1, pp. 31-36.

HELMREICH, F. H. (1929). Factors influencing deposition of fat on the hog. *Am. Soc. An. Prod.* Ann. Meeting, 1929, pp. 143-145.

HELSER, M. D. (1930). Influence of the animal's age upon the quality and palatability of beef. I. Carcase yields and quality of meat cuts. *Iowa Sta. Bull.* 272, pp. 301-311.

HERTER, M., AND WILSDORF, G. (1914). Die Bedeutung des Schweines für die Fleischversorgung. *Arb. der Deutschen Landw.-Gesellschaft*, No. 270.

HUSZTI, D. (1930). Gewichtsänderung der ungarischen Mangaliczasauen während der Zeit der Trächtigkeit und des Säugens; Entwicklung der in verschiedenen Alter entwöhnten Ferkel. *Közl. az Osszehasonlits Elet es Kortan Köreböl*, Vol. 22, Nos. 1-3. Abstr. in *Zuchtungskunde* (1930), Vol. 5, No. 9, p. 407.

IMP. EC. COM. (1929). Pigs and pig products. 12th Report of the Imperial Economic Committee. H.M. Stat. Office, London.

JESPERSEN, J., AND MADSEN, M. P. O. (1931). Beretning om Afkoms-undersögelse over orner af Dansk Landrace. Issued by De Samvirkende Danske Andelsslagterier, Copenhagen.

JOHANSSON, IVAR (1931). Problems in breeding for high prolificacy. *Pig Breeders' Annual*, Vol. 11, pp. 80-87.

KEITH, T. B. (1930). Relation of size of swine litters to age of dam and to size of succeeding litters. *Jnl. Agric. Res.* (U.S.A.), Vol. 41, No. 8, pp. 593-600.

KELLEY, R. B. (1932). The pig industry. *Commonwealth of Australia, Pahphlet No.* 28, pp. 1-43.

KRALLINGER, H. (1930). Über einige das Geschlechtsverhältnis beeinflussende Faktoren. *Züchtungskunde,* Vol. 5, No. 11, pp. 490-501.

KULOW, H. (1929). Ein Vergleich zweier Blutlinien von Wurfgeschwistern des deutschen veredelten Landschweines in bezug auf Körperformen und Leistungen. *Zeitsch. f. Tierzüchtung u. Züchtungsbiologie,* Vol. 13, pp. 1-92.

LARSSON, S. (1928). Die Einwirkung des Mästungsgrades auf der Futterverbrauch der Schweine pro Kg. zuwachs. Mitteilungen No. 338 der Zentralanstalt für Landwirtschaftliches Versuchswesen, Stockholm. Abstr. in *Züchtungskunde,* Vol. 4, No. 2, p. 95.

LUSH, CULBERTSON and HAMMOND (1931). Weight at birth important in pigs. *Poland China Jnl.,* Vol. 18, No. 4, Abstr. in *Zuchtungskunde* (1932), Vol. 7, No. 5, p. 194.

MARTIN, E. (1928). Soft pork investigations. *Ark. Sta. Bul.* 231, pp. 42-44. Abstr. in *Expt. Stn. Record* (1929), Vol. 60, p. 857.

MENZIES KITCHIN, A. W. (1930). East Anglian Pig Recording Scheme. Third report, July, 1930, pp. 1-36.

MENZIES KITCHIN, A. W. (1931). East Anglian Pig Recording Scheme. Report for the year April 1st, 1930, to March 31st, 1931. Unpublished m.s.

MORRIS, H. P., AND JOHNSON, D. W. (1932). Effects of nutrition and heredity upon litter size in swine and rats. *Jnl. Agric. Res* (U.S.A.), Vol. 44, No. 6, pp. 511-522.

MOSSE, K., AND BRAHM, C. (1929). Der Einfluss der Ernährung auf die Zusammensetzung des Fettgewebes. *Jb. Kinderheilk.,* Vol. 122. Abstr. in *Zuchtungskunde* (1931), Vol. 6, No. 5, p. 200.

MOULTON (1929). Meat through the microscope. Chicago.

OLOFSSON, N. E., AND LARSSON, S. (1930). Undersökningar angaende Modersuggornas Produkten och Smägrisarnas Utvveckling. *Centralanstaltens Meddelande* No. 371.

OLSON, F. C., AND BULL, S. (1931). Effect of sex on yield of pork products. *Nat. Provisioner,* Vol. 85, No. 18, pp. 17, 18, 43. Abstr. in *N.P.B.A. Gazette,* No. 20, 1932, p. 53.

ONG, V. S. (1929). A comparative study of the cost of growing and fattening barrows, spayed females and gilt pigs for market. *The Philippine Agriculturist,* Vol. 18, No. 4.

RICHTER AND OTHERS (1928). Untersuchungen an Sauen und Ferkeln während der Saugezeit bei den wichtigsten deutschen Schweinerassen. *Arbeiten der Deutsche Gesell. fur Zuchtungskunde,* No. 37.

ROMYN, A. E., AND OTHERS (1930). Investigations in to the production of bacon. *16th Report of the Dir. of Vety. Serv. & Anim. Indust.* U. of South Africa. pp. 503-522.

SALMON, W. A. (1927). Effect of starvation period on the firmness of carcase. *Ala. Sta. Rpt.,* pp. 11-18.

SCHMIDT, J., AND VOGEL, H. (1930). Bericht über die Schweineleistungsprüfungen in dem Provinz Hannover. *Zuchtungskunde,* Vol. 5, No. 5, pp. 193-215.

SCHMIDT, J., AND VOGEL, H. (1931). Untersuchungen uber die Gewichte von innere Organen bei Mastschweinen und ihre Beziehung zu Leistung und Körperform. *Zuchtungskunde,* Vol. 6, No. 6, pp. 224-231.

SCHMIDT, J., VOGEL, H., AND ZIMMERMANN, C. (1929). Leistungsprüfungen. *Arbeiten der Deutschen Gesellschaft fur Züchtungskunde,* No. 47.

SCHMIDT, J., AND OTHERS (1931). Über die Zusammensetzung des Schweinekörpers. *Wiss. Archiv. fur Landw. Abt. B,* Vol. 5, No. 2, pp. 237-375.

SCHUTTE, D. J., AND MURRAY, C. A. (1931). The effect of barley in the ration on the quality of bacon. *17th Rept. of the Dir. of Vety. Serv. & Anim. Indust.*, Union of S.A., pp. 813-817.

SCOTT, E. L. (1929). Factors that influence the significance of hog carcase data. *Am. Soc. An. Prod.*, Proc. Ann. Meeting, 1929, pp. 136-142.

SHAW, A. M. (1929). Variations in the skeletal structure of the pig. *Scientific Agriculture*, Vol. 10, No. 1.

SPÖTTEL, W. (1932). Der Einfluss der Fütterung auf die Körperform, die Organe und Leistung der Schafe. *Züchtungskunde*, Vol. 7, No. 4, pp. 117-138.

STAHL (1930). Ruhlsdorfer Beobachtungen zur Ferkelaufzucht. *Zeits. fur Schweinezucht*, Nos. 26, 27 and 28.

TEMPEL, W. (1931). Beitrag zur Feststellung der Gewichtsverhältnisse der Fleischqualitäten beim Rind unter besonderer Berücksichtigung des Chemnitzer Fleischergewerbes. Inaug. Diss. Leipzig.

WARWICK, B. L., AND VAN LONE, E. E. (1926). The effect of castration on pigs at different ages. *Jnl. Am. Vet. Med. Ass.*, Vol. 69, N.S. 22, No. 5.

WELLMANN, O. (1930). Nährstoffbedarf junger schweine auf Grund von Stoffwechseluntersuchungen und Ferkelanalysen. *Mezögazdasagi Kölony*, Jg. 3, p. 82. Abstr. in *Züchtungskunde* (1930), Vol. 5, No. 11, p. 513.

WENCK, E. (1931). Über die Beziehung zwischen der Entwicklung der Saugferkel und ihren späteren Mastleistungen als Grundlage der Herden—und Zuchtwertbeurteilung. *Zeits. fur Tierzuchtung und Zuchtungsbiologie*, Vol. 22, No. 1, pp. 1-33.

WENTWORTH, E. N., AND LUSH, J. L. (1923). Inheritance in swine. *Jnl. Agric. Res.* (U.S.A.), Vol. 23, No. 7, pp. 557-582.

WILD, H. (1927). Ergebnisse von Schweineleistungsprüfungen, insonderheit Studien über die Ferkelentwicklung. Inaug. Diss. Berlin. Abstr. in *Züchtungskunde* (1929), Vol. 4, No. 10.

WILKENS, C. (1929). Über Zucht und Wachstum des deutschen veredelten Landschweines im Regierungsbezirk Lüneburg. *Züchtungskunde*, Vol. 4, No. 8, pp. 380-392.

WILSON, W. K., AND MORRIS, S. (1932). Studies in the composition of rabbit carcasses. 1. White Angoras. *Jnl. Agric. Sci.* (Eng.), Vol. 22, Part 3, pp. 453-459.

ZORN, W., AND HEIDENREICH, C. H. (1930). Die Begutachtung der Fleischfarbe bei Versuchsschlachtungen von Schweinen. *Wiss. Archiv. fur Landw.* Abt. B, Vol. 3, No. 1.

ZORN, W., AND KRALLINGER, H. F. (1930). Grundsätzliches über haustiergenetische Forschung. *Landw. Jb.*, Vol. 71. Abstr. in *Züchtungskunde* (1931). Vol. 6, No. 5, p. 192.

Studies on the Origin of the Sulphur in Wool.

I. A Study of the Sullivan Technique for Cystine.

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THE solution of biological and nutritional problems centering around sulphur metabolism has been appreciably advanced, since Sullivan's (1926) discovery that the reaction with 1,2-Naphthoquinone-4-Sodium sulphonate under certain conditions constitutes a highly specific reaction for cysteine or cystine. Admittedly, the other methods such as the iodometric methods of Okuda (1925) and of Baernstein (1930), and the colorimetric method of Folin and Marenzi (1929) may sometimes be applied with advantage when dealing with relatively pure solutions of amino-acids or protein hydrolysates; their non-specificity, however, limits their general application, especially when dealing with more complex biological material.

The accuracy of the Sullivan technique, as a quantitative method, has however been criticised by various workers, and it is to be admitted that such criticism has for the greater part shown itself to be justified. The Sullivan technique as described by Sullivan himself contains several weaknesses and inherent errors. To circumvent these difficulties various modifications of Sullivan's original procedure have been suggested. Apparently none of these suggested modifications can be claimed to be based on a systematic study of the Sullivan technique. In the present paper an endeavour will be made to give experimental data to indicate along what lines the Sullivan procedure may be modified with advantage. On the other hand, certain objections to the method, such as the interference of other amino acids, radicles, ions and adventitious colouring matter, cannot be avoided by modification of the method as such. This aspect of the matter will be dealt with in a later publication, when a new micro method for the determination of cystine or cysteine in biological matter, depending on the quantitative isolation of cystine as cysteine cuprous mercaptide with subsequent determination of the cystine by the Sullivan method, will be described.

The chief difficulty with the original Sullivan method would seem to lie in the application of the cysteine reaction to the determination of cystine. Sullivan (1926) seems to have come to the conclusion that the degree of correspondence of colour intensity between cystine and cysteine varied with cystine concentration, and that the reduction of cystine indicated only 50-75 per cent. of the theoretical. This statement would appear to have caused considerable confusion. It must not be taken to signify that up to 75 per cent. of a given amount of cystine can be transformed to cysteine by the action of cyanide or sulphite. As Pulewka and Winzer (1928) have shown, one molecule of cystine reacts with cyanide to form only one molecule of cysteine, according to the equation:—

$$ R - S - S - R \ + \ NaCN \ \rightarrow \ R - SNa \ + \ R - SCN $$
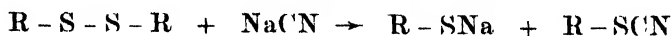
Similarly Clarke (1932) has shown that from one molecule of cystine only one molecule of cysteine is obtained by the action of sulphite:—

$$ R - S - S - R \ + \ Na_2SO_3 \ \rightarrow \ R - SNa \ + \ R - SSO_3Na $$

It is clear, therefore, that reduction by this means cannot transform more than 50 per cent. of the cystine to cysteine. Sullivan's findings can therefore be interpreted only as signifying that the above reactions proceeded to the extent of 50 to 75 per cent., i.e. that one molecule of cystine produced only 0·5 to 0·75 of a molecule of cysteine.

In this connection it is interesting to note that Lugg (1933) states that, even if the conditions be varied considerably, the above reaction with cyanide proceeds so nearly to completion that any deviation from it cannot be detected colorimetrically. Lugg gives no details as to which conditions may be varied, but states further on in his paper that if the amount of cyanide is halved, a 25 per cent. loss in colour intensity occurs, while if the cyanide be doubled or trebled the final colour gives a transient brown on agitation in air.

It is therefore necessary that we should consider the influence of cyanide concentration on the reduction and resultant colour development more carefully. To 0·25 mg. and 0·50 mg. respectively of cystine contained in suitable aliquot volumes, increasing amounts of cyanide were added, the volumes of the mixtures being kept constant at 9·0 c.c. After 10 minutes 1·0 c.c. of 0·5 per cent. of the fresh naththoquinone reagent was added, the solution well mixed, and 25 seconds later 5 c.c. of 10 per cent. anhydrous Na_2SO_3 in 0·5 N NaOH solution added. The solution was mixed and left to stand for 30 minutes. Finally 1·0 c.c. of a 1-2 per cent. $Na_2S_2O_4$ in 0·5 N NaOH solution was added, and the solutions compared colorimetrically, using the solution containing 2·0 c.c. 5 per cent. NaCN as standard. The results have been represented graphically in Fig. 1.

Fig. 1.

EFFECT OF NaCN ON COLOUR DEVELOPMENT.
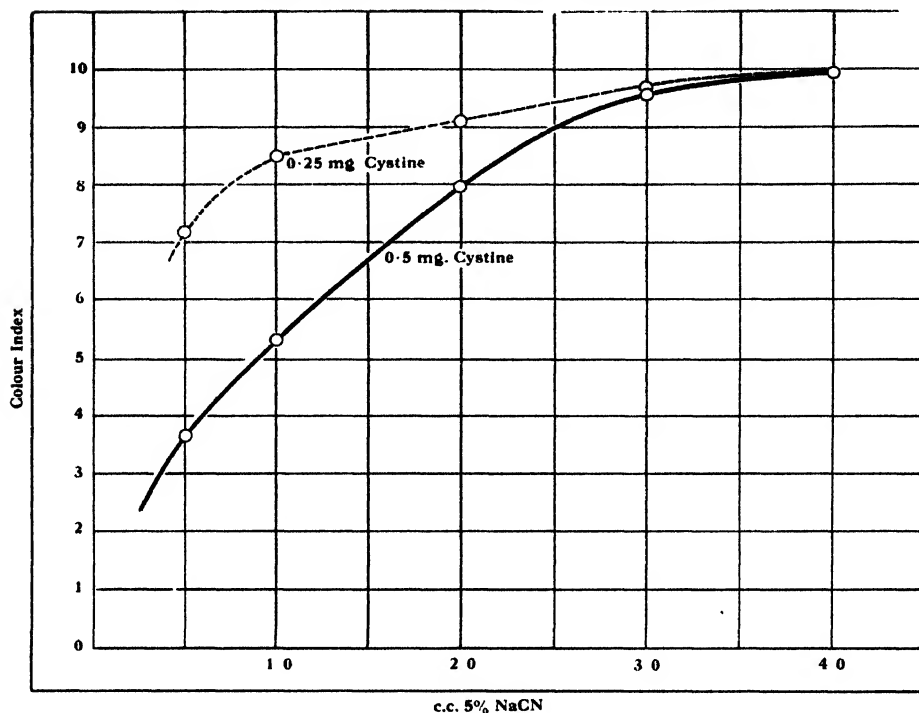
As will be seen from the figure, the curves for both the 0·25 and 0·50 mg. cystine show a strong tendency to run horizontally when a cyanide concentration of 4·0 c.c. 5 per cent. NaCN per 9·0 c.c. solution is reached.

In order to show that at this cyanide concentration the reaction

$$R - S - S - R + NaCN \rightarrow R - SNa + R - SCN$$
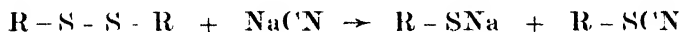
had, for all practical purposes, attained completeness, a cystine standard containing 0·40 mg. cystine was compared colorimetrically with two cysteine standards each containing 0·205 mg. of cysteine, the one treated precisely in the same way as the cystine standard, and the other according to the Sullivan procedure for cysteine (with the exception that the time factor of 25 seconds was observed). No measurable difference in colour intensity of the three solutions could be obtained. In addition to this it was shown that for this higher cyanide concentration a variation in the time interval from 5 to 15 minutes produced no measurable effect on the ultimate colour intensity.

If, then, the addition of 4·0 c.c. 5 per cent. NaCN liberates 1 molecule of cysteine from one molecule of cystine, Sullivan's findings with 1·0 or 2·0 c.c. 5 per cent. NaCN may easily be explained. As the 0·5 mg. cystine curve shows, the use of 1·0 c.c. cyanide would

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result in the liberation of just over 0·5 molecule cysteine, whereas the use of 2·0 c.c. cyanide yields about 0·8 molecule cysteine. In the 0·25 mg. cystine curve the cysteine yields for 1·0 and 2·0 c.c. cyanide are respectively appreciably higher. Thus, when working under conditions of cyanide deficiency, Sullivan is essentially correct in stating that the correlation in colour intensity between cystine and cysteine depends on the amount of cystine present. Theoretically, of course, any of the cyanide concentrations mentioned should lead to correct assays when all the material is present as cystine, provided both assay and standard are treated in exactly the same way. In practice, however, the difficulty of duplicating any set of conditions increases with the steepness of the curve, and for this reason alone it would be desirable to work at a cyanide concentration represented by 4·0 c.c. 5 per cent. NaCN, or what is more practical, 2·0 c.c. 10 per cent. NaCN.

Apart from the influence of cyanide concentration on the completeness of the cyanide-cystine reaction, there is another step in the Sullivan procedure which may lead to serious errors. Sullivan gives no indication that the time interval between the adding of the naphthoquinone reagent and the alkaline sulphite must be carefully controlled. In Fig. 2 the colour intensity has been plotted against this time interval in seconds, using 0·40 mg. of cystine. Both the 2·0 c.c. 10 per cent. NaCN curve and the 2·0 c.c.

Fig. II.

EFFECT OF NAPTHOQUINONE SULPHITE.

Time Interval on Colour Development.
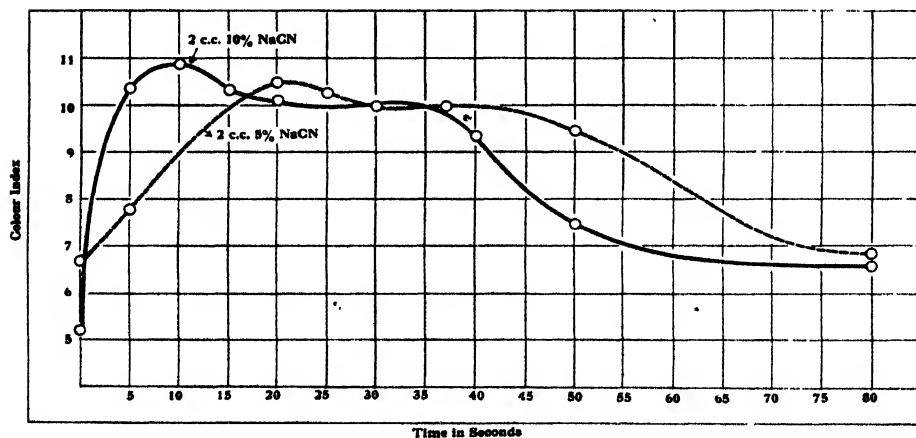
5 per cent. NaCN curve show the same peculiar form; the only difference would appear to lie in the acceleratory influence of the higher cyanide concentration, thereby shifting the higher cyanide curve to the left. It should be observed, however, that when the naphthoquinone-sulphite time interval is below a certain minimum, depending on cyanide concentration, the curves are steep. Thus, if we were to adopt Sullivan's original procedure of adding the sulphite

immediately after adding the naphthoquinone and mixing, we would invariably be working on this steep part of the curve. It is due mainly to the failure of Sullivan to stipulate the control of this time factor, that his method, as applied to relatively pure solutions has been found to give irregular results, as stressed by Rimington (1929), Prunty (1933), and Lugg (1933).

Apparently Csonka (1932) was the first to suspect the existence of a time factor at this stage of the process, since in his modification of the Sullivan technique he stipulates that after adding the naphthoquinone reagent the solution be shaken for 10 seconds before adding the alkaline sulphite. Lugg (1933) on the other hand employed a time interval of no less than 5 minutes. Although it would appear that Lugg was working on a fairly horizontal part of the curve, the employment of a 5-minute time interval is not to be recommended, chiefly for two reasons. In the first place Lugg was working under conditions where the final colour intensity fell far below the optimum maximum intensity; and in the second place his 5-minute time interval would seem to make the whole process too susceptible to variations in cyanide concentrations. In this connection it should be observed that Lugg adds $2 \cdot 0$ c.c. of molar or $4 \cdot 9$ per cent. NaCN solution to a mixture of about 16 c.c. of cystine and buffer mixture. Under such conditions it is inconceivable that one molecule of cysteine can be freed from one molecule of cystine, unless the buffer solution exercises a strongly positive catalytic acceleration of the reaction. In spite of this Lugg reports that doubling or trebling the amount of cyanide adversely affects the final colour tint obtained.

In contrast to Lugg's observations it was found that the colour tint is in no way impaired by adding $2 \cdot 0$ c.c. 10 per cent. NaCN to $5 \cdot 0$ c.c. cystine solution, provided the time interval between naphthoquinone and sulphite is regulated to 20-30 seconds. As a matter of fact the choice of this time interval is strongly suggested by the curve in Fig. 2, as representing the most suitable conditions for obtaining the optimum maximum colour development.

The method as ultimately adopted in this laboratory is as follows: —

> 5 c.c. of standard cystine solution, slightly acid with HCl and containing $0 \cdot 4$ mg. of cystine.
>
> 2 c.c. 10 per cent. aqueous solution of NaCN. Mix and wait 10 minutes.
>
> 1 c.c. of a $0 \cdot 5$ per cent. naphthoquinone solution. Mix and allow 20 seconds.
>
> 5 c.c. 10 per cent. sodium sulphite in $0 \cdot 5$ N NaOH. Mix and allow 30 minutes.
>
> 1 c.c. 1-2 per cent. sodium hydrosulphite solution in $0 \cdot 5$ N NaOH.

Mix and compare colours after being left in the colorimeter cups for about three minutes. All the reagents must be freshly prepared, especially the naphthoquinone and the sodium hydrosulphite.

Apart from the factors already discussed various other objections have been raised against the Sullivan method. For the greater part all these objections centre around the influence of substances and ions other than cystine in solution on the ultimate intensity of colour development.

Thus Lugg (1933) states that the presence of other amino acids in relatively large quantities diminish the colour obtained, while the yellow colour given by these amino acids after addition of hydrosulphite ($Na_2S_2O_4$) tends to interfere with the colorimetric comparison. This interference on the part of other amino acids has been verified here, as the results in the table below clearly show.

Influence of Amino Acids on Cystine Colour Development.

Amino Acids + 0·4 mg. Cystine.	Percentage Normal Colour.	
	50 × wt. of Cystine.	100 × wt. of Cystine.
Glutamic acid hydrochloride	97	—
Histidine hydrochloride	—	95
Alanine	—	94
Tyrosine	87	83
Tryptophane	90	81
Aspartic acid	85	71

Thus Lugg in his method " swamps " both assay and standard with glycine. The soundness of the principle of such a procedure would seem rather doubtful, since it involves the adding of an excess amount of interfering material in order to counterbalance the influence of interfering material already present.

Apart from the presence of other amino acids, the solutions in which the cystine is to be determined usually contain smaller or larger amounts of sodium chloride or sulphate depending on the cystine content of the original material and the amount of acid used for hydrolysis. In one of his later publications Sullivan (1929) notes that the larger 'amounts of sodium chloride may lead to appreciable errors. In this connection it would appear that sodium chloride has both an acceleratory and intensifying action. According to the curves in Fig. 3 the action of sodium chloride is not very marked when employing 2·0 c.c. 10 per cent. NaCN for the reduction of cystine to cysteine.

Fig. 3.

INTENSIFYING EFFECT OF NaCl.
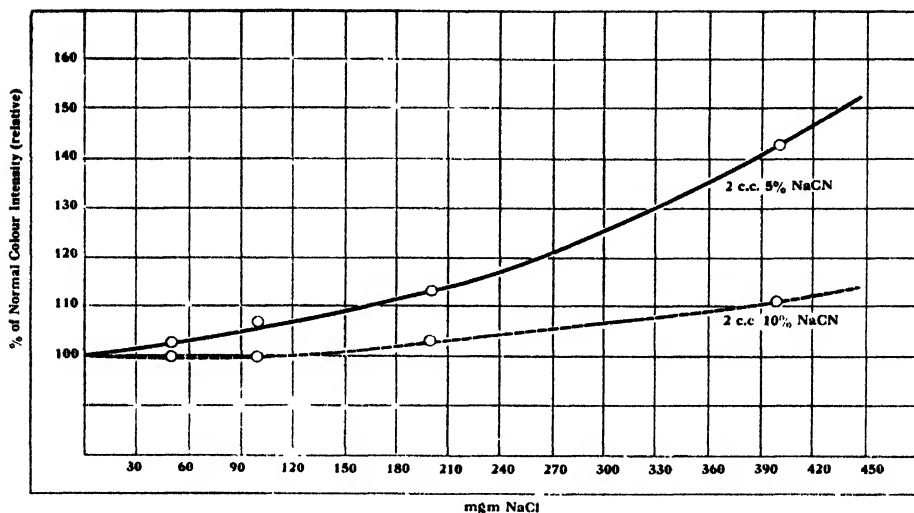
Since with such cyanide concentrations the cystine-cyanide double reaction is practically complete, the observed effect of the sodium chloride must be ascribed to its intensifying action. On the other hand, the 2·0 c.c. 5 per cent. NaCN curve indicates that under such conditions the sodium chloride effect is fairly marked at higher concentrations. Since the cystine-cyanide reaction under conditions of cyanide deficiency has been shown not to be complete, the sodium chloride in this case would seem to have an acceleratory as well as intensifying action. Incidentally this appreciable difference in the influence of sodium chloride as exhibited by the two curves in Fig. 3, illustrates another disadvantage in the original Sullivan technique.

The interference of inorganic salts is, however, not limited to sodium chloride; it has been found that sodium sulphate, potassium sulphate and potassium chloride produce a similar effect, although to a somewhat lesser extent. Lugg has shown that the presence of ferric iron in appreciable concentrations may lead to serious errors. The same was found to apply to zinc. The suggestion made by Prunty (1933) first to reduce the cystine to cysteine by means of zinc dust must, therefore, be considered with the greatest caution. According to our own experiments, minute amounts of zinc seem to exercise no measurable influence; on the other hand, larger amounts of zinc cause turbidity and produce unreliable colours. As an example of a strong inhibitor sodium borate may be mentioned, 60 mg. with 0·4 mg. cystine producing only 64 per cent. of the normal colour development. More remarkable still is the effect of ammonium salts. On adding 400 mg. of ammonium sulphate to 0·4 mg. cystine a perfect blank was obtained on the subsequent addition of the hydro-sulphite; 100 mg. showed a recovery of 56 per cent. and even 10 mg. still showed a loss of 10 per cent. Ammonium chloride produced the same effect.

As most protein hydrolysates will contain ammonium salts in small and even considerable quantities, this shows a further disadvantage of the Sullivan method when applied directly.

In view of the interference so far established, the direct applicability of the Sullivan method to all types of hydrolysates and other solutions would seem to be open to serious doubt. The great specificity of the reaction as shown by Sullivan merely indicates that no other substance is known to give the reaction by itself, yet such a finding in no way proves that the same substance may not appreciably influence the colour reaction when present together with cystine. As far as is known, this latter type of interference is to a greater or lesser degree common to most colorimetric methods.

SUMMARY.

It has been shown that the Sullivan reaction as a quantitative colorimetric method for the determination of cystine can be considerably improved by better regulation of the conditions necessary for optimum maximum colour development. This improvement has been brought about, chiefly by increasing the cyanide concentration and regulating the time interval between the adding of the napthoquinone reagent and the alkaline sulphite.

It has been found that various substances interfere with the intensity of the final colour obtained. Thus other amino-acids, ammonium salts and sodium borate have been shown to retard or inhibit the reaction, while sodium and potassium chlorides and sulphates have been found to exercise an acceleratory and intensifying action.

REFERENCES.

BAERNSTEIN, H. D. (1930). The Gasometric Determination of Cysteine and Cystine. *J.B.C.*, Vol. 89, p. 128.

CLARKE, H. T. (1932). The Action of Sulphite upon Cystine. *J.B.C.*, Vol. 97, p. 235.

CSONKA, F. A. (1932). Studies on Glutelins, VII. Cystine, Tryptophane, and Tyrosine Content of Glutelins. *J.B.C.*, Vol. 97, p. 281.

FOLIN, O., AND MARENZI, A. D. (1929). An Improved Colorimetric Method for the Determination of Cystine in Proteins. *J.B.C.*, Vol. 83, p. 103.

LUGG, J. W. H. (1933). Sullivan's Reaction for the Quantitative Determination of Cysteine and Cystine. *Bioch. Jnl.*, Vol. 27, p. 668.

OKUDA (1925). *J. Biochem.* (Tokio), Vol. 5, p. 217. See Prunty (1933).

PRUNTY, F. T. G. (1933). The Determination of Cystine in Biological Material. *Biochem. Jnl.*, Vol. 27, p. 387.

PULEWKA AND WINZER (1928). Untersuchungen über die Reaktionen des Cystins mit Schwefel-und Cyanalkali. *Arch. Exp. Path. u. Pharmakol*, Bd. 138, s. 154.

RIMINGTON, C. (1929). The Relation between Cystine Yield and Total Sulphur in Wool. *Biochem. Jnl.*, Vol. 23, p. 41.

SULLIVAN, M. X. (1926). A Distinctive Test for Cysteine. *U.S. Publ. Health Reports*, 28th May, 1926, p. 1030.

SULLIVAN, M. X. (1929). Studies on the Biochemistry of Sulphur, IV. The Colorimetric Estimation of Cystine in Casein by Means of the Beta-Naphthoquinone Reaction. *Supplement No. 78 to U.S. Publ. Health Reports* (1929).

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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1908-9.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1909-10.*

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Second Report of the Director of Veterinary Research, October, 1912.*

Third and Fourth Reports of the Director of Veterinary Research, November, 1915.*

Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918.*

Seventh and Eighth Reports of the Director of Veterinary Research, April, 1918.*

Ninth and Tenth Reports of the Director of Veterinary Education and Research, April, 1923.

Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part 1, September, 1926.

Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part II, January, 1927.

Thirteenth and Fourteenth Reports of the Director of Veterinary Education and Research, Parts I and II, October, 1928.

Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.

Sixteenth Report of the Director of Veterinary Services and Animal Industry, August, 1930.

Seventeenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1931.

Eighteenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1932.

Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 1, June, 1933.

Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 2, October, 1933.

Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. II, No. 1, January, 1934.

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Director of Veterinary Services and Animal Industry.

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Section I.

Virus Diseases.

The Immunization of Horses and Mules against Horsesickness by means of the Neurotropic Virus of Mice and Guinea-Pigs.

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IN December, 1932, Nieschulz reported that he had succeeded in infecting white mice with the virus of horsesickness by the intracerebral route. Independently and concurrently this work was confirmed at Onderstepoort (Alexander, 1933), but in one important respect the conclusions differed from those arrived at by Nieschulz. Whereas Nieschulz maintained that the virus was not attenuated and that its character was not altered by passage through the mouse, our experience with a greater number of horses showed that not only was there an attenuation of the virus for the horse, but that, after even a limited number of generations, the change·was so marked that there existed a distinct possibility of developing a safe and simple method of immunization against horsesickness in a manner similar to that applied to yellow fever by Sawyer and his co-workers.

With this object in view the virus has been passaged serially in mice, so that, at the time of writing, one strain is in its 89th generation, a second antigenically different strain is in its 78th generation, and a third in its 64th generation. In addition to the propagation in mice the first two of the above-mentioned strains have been maintained in guinea-pigs, in which animal they have been passaged 38 times.

As reported previously, in both these animals the virus adopts exclusively neurotropic characters. After 2 to 4 subinoculations a mortality of 100 per cent. is produced, and there is a progressive acceleration of the course of the disease. From time to time susceptible horses and mules have been injected with emulsions of virulent brains in order to ascertain the degree of attenuation for equines. The results obtained form the basis of this report.

Virus Used.—In the experiments reported two antigenically different strains of virus have been used.

(1) Strain O (ordinary virus, known as O virus), an extremely virulent strain isolated some 30 years ago by Sir Arnold Theiler and maintained by periodical subinoculation into horses. Generation 193 was used in the form of whole blood drawn from a reacting horse (20319) at the height of the febrile reaction into oxalate-carbolglycerin solution as an anticoagulant and preservative. Stored in a cool room in this way the blood is known to retain full virulence for years.

(2) Strain 20449 obtained in 1932 from a fatal case of horse-sickness that occured in an animal that had been hyperimmunized some months previously against strain O by the transfusion of 10 litres of virulent blood. It has been shown experimentally that this strain breaks down the immunity set up by O virus, and conversely horses immunized against 20449 strain always react to a subsequent intravenous injection of O virus.

Behaviour of the Strains in Mice and Guinea-Pigs.—At this stage it is unnecessary to describe and contrast the behaviour of these strains in mice and guinea-pigs. It is sufficient to state that in the case of O virus the course of the disease in mice has gradually decreased from 5-7 days to 4 days after 75 passages, and that 0·05 c.c. of a 1-100,000 dilution of fresh brain represents approximately 1 lethal dose. In guinea-pigs death usually occurs in 6-8 days, and the infective titre of the brain is about 1/10 that of mice.

Strain 20449 invariably kills 100 per cent. of injected mice on the 4th day, and emulsions of fresh brains have frequently proved infective in a dilution of 1:1,000,000. In guinea-pigs the course of the disease is somewhat longer than that produced by O. virus, but the infectivity appears to the approximately the same.

PREPARATION OF VIRUS EMULSION FOR INJECTION.

Infected mice and guinea pigs are etherized *in extremis*, the brains removed with complete aseptic precautions, and placed in sterile 50 c.c. centrifuge tubes fitted with sterile corks. The brains are then frozen overnight in the freezing chamber of an electric refrigerator and next morning rapidly thawed in a water bath or incubator at 37° C. to cause the disintegration of as much cellular material as possible. The amount of fluid necessary to give the desired concentration (usually 4 per cent.) is introduced, and the brain material is macerated by rapidly drawing it into, and forcing it out of, a syringe fitted with a fine nozzle. The resulting emulsion is then centrifuged for 20 minutes at ±1,500 revolutions per minute, and the turbid supernatant fluid free from gross particulate matter is used for injection. In the text this fluid is referred to as " virulent brain emulsion ".

For emulsification serum of a normal susceptible horse diluted 1:10 with 0·85 per cent. saline was used, since it has been found to have several decided advantages over saline alone.·

In those experiments where various dilutions of infective brain material were used, a stock emulsion representing a dilution of 1 part of brain substance to 25 parts of 1:10 serum-saline was made, and the requisite dilutions in serum-saline were prepared from this stock.

HORSES AND MULES USED.

The horses and mules used were obtained from various horse dealers, who purchase their animals in districts where horsickness normally does not occur. These dealers have supplied this Institution for many years, and experience has shown that only on rare occasions have immune animals been included in a batch. The percentage of such animals which were immune as a result of recovery

from a natural attack of horsesickness has never exceeded 2 per cent. Consequently it must be pointed out that although it is possible for an immune individual to be drafted into an experiment, duplication of each experiment will certainly prevent the drawing of erroneous conclusions from the use of insusceptible animals.

A. THE IMMUNIZATION OF HORSES.

The first experiment to ascertain the antigenic value of mouse neurotropic virus was carried out on two horses. The entire emulsion of the brain of one mouse, generation 5, strain 20449, was injected subcutaneously into horse 20337 on 22/11/32. There was no local reaction at the site of injection, but a mild systematic reaction characterized by slight fever commenced on the 6th day after injection and lasted for 5 days. The horse recovered rapidly and at no time would it have been possible for a clinical diagnosis of horsesickness to have been made.

Subsequently, on 6/12/32, an emulsion of the pooled brains of 9 mice, generation 8, strain 20449, was made in 100 c.c. serum-saline; 50 c.c. were injected subcutaneously into the same horse; and 50 c.c. intravenously into a 2nd horse (20334). Horse 20337 did not react. Horse 20334 developed a fairly severe febrile reaction which lasted from the 5th to the 10th day, accompanied by marked oedema of the supraorbital fossa (dikkop) and recovered.

Fourteen days later (20/12/32) both horses were given 5 c.c. of the homologous strain of virulent blood intravenously as an immunity test; neither horse reacted. That this blood was fully virulent was shown by the fact that a control horse commenced to react on the 3rd day after injection and died on the 6th day.

In passing it may be mentioned that on 3/1/33 both the above horses were given 5 c.c. of virus, strain O, intravenously. Horse 20337 reacted and died on the 6th day; horse 20334 underwent a very severe reaction, but eventually recovered.

From this experiment it must be concluded that during the process of transformation from viscerotropism to neurotropism there is a marked alteration in the virulence of the virus for horses. Consequently utilization of this process of natural attenuation encouraged the hope that after complete " fixation " the neurotropic virus might serve as a vaccine, since there did not appear to be any decrease in antigenic activity.

To confirm the conclusions drawn from this initial experiment on 2 horses, a second experiment was commenced, with the object of—

    (*a*) ascertaining whether even a limited number of passages through the brains of mice and guinea-pigs would attenuate the virus to a consistently safe level;

    (*b*) determining the most suitable route for the injection of equines, i.e. subcutaneous or intravenous;

    (*c*) comparing the relative efficacy and degree of attenuation of the mouse and guinea-pig adapted viruses respectively.

The injections given and the results obtained are indicated below : —

20492. 28/12/32. Subcut. 50 c.c. emulsion 3 mouse brains, generation 12 and 13, strain 20449. Severe reaction commenced 6th day. Died 11th day. Dikkop horsesickness.

20493. 28/12/33. Intravenously 50 c.c. emulsion 3 mouse brains, generation 12 and 13, strain 20449. Slight febrile reaction from 6th to 11th day. Recovered.

*16/1/33. Intravenously 5 c.c. virulent blood strain 20449. No reaction.

25/4/33. Subcutaneously 10 c.c. 1/25 dilution 1 brain mouse, gm. 29, strain O. Severe reaction from 7th to 14th day. Recovered.

(NOTE.—H. 20493 had an accident and had to be destroyed before the application of an immunity test to O virus.)

20494. 28/12/33. Intravenously 50 c.c. emulsion 1 guinea-pig brain, generation 3, strain 20449. Mild reaction from 6th to 11th day. Recovered.

*16/1/33. Intravenously 5 c.c. virulent blood strain 20449. No reaction.

13/2/33. Subcutaneously 50 c.c. emulsion 2 guinea-pig brains, generation 5, strain O. No reaction.

*3/3/33. Intravenously 5 c.c. virulent blood strain O. No reaction.

20495. 28/12/32. Subcutaneously 50 c.c. emulsion 1 guinea-pig brain, generation 3, strain 20449. No reaction.

*16/1/33. Intravenously 5 c.c. virulent blood strain 20449 No reaction.

30/1/33. Subcutaneously 15 c.c. emulsion 1 mouse brain, generation 13, strain O. Severe reaction from 3rd to 13th day. Recovered.

*20/2/33. Intravenously 5 c.c. virulent blood, strain O. No reaction.

20496. 30/12/32. Subcutaneously 15 c.c. emulsion 3 mouse brains, generation 7, strain O. Severe reaction commenced 3rd day. Died 9th day.

20497. 30/12/32. Intravenously 15 c.c. emulsion 3 mouse brains, generation 7, strain O. Reacted from 4th day. Died 8th day.

20502. 18/1/33. Intravenously 10 c.c. emulsion 1 guinea-pig brain, generation 5, strain 20449. Mild fever reaction from 8th to 14th day.

*10/2/33. Intravenously 5 c.c. virulent blood strain 20449. No reaction.

2/3/33. Subcutaneously 10 c.c. 1/500 dilution 1 mouse brain, generation 16, strain O. Severe reaction from 5th day. Died 9th day.

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* Denotes immunity test.

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20503.	18/1/33.	Intravenously 10 c.c. emulsion 1 guinea-pig brain, generation 5, strain 20449. Mild fever reaction from 9th to 17th day.
	*10/2/33.	Intravenously 5 c.c. virulent blood, strain 20449. No reaction.
	2/3/33.	Subcutaneously 10 c.c. 1/500 dilution 1 guinea-pig brain, generation 6, strain O. Severe reaction from 5th day. Died 10th day.
20538.	22/2/33.	Subcutaneously 50 c.c. 1/100 dilution 1 guinea-pig brain, generation 5, strain O. Severe reaction from 2nd day. Died 6th day.

RESULTS AND CONCLUSIONS.

(1) There appears to be no significant difference in the results obtained from the injection of the virus emulsion subcutaneously or intravenously. In the first instance (horses 20492 and 20493) the subcutaneous injection produced the more severe reaction; in the second instance (horses 20494 and 20495) the more severe reaction was a result of the intravenous injection; in the third instance (horses 20496 and 20497) both the injected animals died. Consequently it was decided that for the sake of ultimate simplicity all injections in the future would be given by the subcutaneous route.

(2) The virus appears to be attenuated more rapidly by serial passage through guinea-pigs than through mice. Whether this attenuation is merely more rapid at the commencement of passage but eventually would attain the same level in both animals after prolonged subinoculation, it is not possible to say at this stage.

(3) The two different strains of virus used did not become attenuated for horses at the same rate as indicated by the fact that horses 20494 and 20495 survived an injection of material consisting of the 3rd generation of strain 20449 in guinea-pigs, while horse 20538, which was injected with emulsion representing the 5th generation of strain O in guinea-pigs, died after an incubation period and duration of illness which indicated that little or no decrease in virulence had taken place. In this connection it is interesting to bear in mind that strain 20449 was obtained from a naturally contracted case of horsesickness, while strain O, which had been passaged for 193 generations in horses before being transferred to guinea-pigs, possibly had become " fixed " for horses.

(4) Apart from the definite attenuation of the virus strains, the most gratifying and most important conclusion that may be drawn from the whole experiment, is that decrease of virulence is not accompanied by a simultaneous loss of any antigenic power. This is clearly illustrated by the observation that every horse which survived an injection of guinea-pig or mouse virus was shown subsequently to be solidly immune to the homologous strain.

* Denotes immunity test.

(5) As regards the immunity produced by one strain of virus (20449) against a second (strain O) no definite conclusion can be drawn, since horses which had survived the injection of 20449 neuro-tropic virus received material representing different generations of strain O, i.e. at different levels of attenuation. It seems probable therefore that if a cross-immunity does exist, it is at most only partial.

The results obtained from the above experiment were so encouraging that it was decided to carry out a further experiment on five horses to determine the degree of attenuation after several additional passages through guinea-pigs and also to ascertain the effect of injecting various dilutions of emulsion.

Three guinea-pigs, constituting generation 10, strain 20449, were destroyed in extremis and a 5 per cent. emulsion of the pooled brains was made in 1/10 normal horse serum-saline. The super-natant fluid obtained after centrifugation was used as the highest concentration of virulent brain, and serial dilutions in serum-saline were made from this stock emulsion. The injections made and the results obtained are tabulated below:—

Horse.	Date.	Dose.	Dilution.	Reaction.
20360	7/3/33	10 c.c. subcut.	1/20	No reaction.
20354	,,	,,	1/100	,, ,,
20371	..	,,	1/500	Reaction commenced 8th day. Died 16th day. Dikkop.
20355	,,	..	1/1,000	Mild febrile reaction	Dikkop from 14–17th day.
20367	,,	..	1/5,000	Reaction commenced 7th day. Died 13th day. Dikkop.

The 3 survivors were given an immunity test of 5 c.c. of virulent blood of the same strain intravenously on 4/4/33. There were no clinical or thermal reactions. A titration of the infectivity of the emulsion for mice was carried out, 0·05 c.c. amounts of each serial dilution in serum-saline being injected intracerebrally into 2 mice.

Dilution.	Result.
1/20	6 : 6*
1/100	6 : 6
1/500	6 : 6
1/1,000	7 : 8
1/5,000	7 : 7
1/10,000	7 : 7
1/50,000	0 : 0

* NOTE.—The numerals indicates the number of days between injection and death of each mouse. 0 means survival.

RESULTS AND CONCLUSIONS.

Of 5 horse injected with the same dose of falling dilutions of neurotropic virus in the 10th passage through guinea-pigs, 2 died of dikkop horsesickness, 1 showed a marked clinical reaction accompanied by oedema of the supraorbital fossa, and 2 did not react. Whereas strain 20449 can be relied upon constantly to produce a reaction by the 4th day and death by the 7th day, the reactions were considerably delayed; in fact, they were of a nature which would have been expected as a result of the simultaneous injection of virus and massive doses of hyperimmune serum. This clearly indicates that a considerable decrease of virulence for horses had taken place, but after 10 generations in guinea-pigs the attenuation had not reached such a level as to permit of the injection into fully susceptible animals with perfect safety.

The 3 survivors, after an interval of 28 days, were solidly immune to the homologous strain of virus, again indicating no diminution of antigenic activity.

A striking feature of the experiment is the fact that the horses which received the lowest concentration of brain substance reacted most severely. If the reactions produced in horses 20355 and 20371 (1 : 1000 and 1 : 500 dilution) were interchanged, there would have been a perfect gradation from no reaction with the highest concentration (1 : 20) to the most severe reaction, with shortest period of incubation and earliest death, with the lowest concentration. The virus titration in mice shows that 0·05 c.c. of a 1 : 50,000 dilution contained less than 1 minimal lethal dose of virus for mice. Therefore the 1 : 5,000 dilution contained between 1 and 10 infective doses and the 1 : 20 dilution between 250 and 2,500 infective doses. Thus the horse (20367) which received a maximum of 2,000 mouse-infective doses reacted more severely than the horse (20360) which received probably 500,000 mouse-infective doses.

This observation appeared to be of such importance that a second experiment was planned on similar lines to confirm the unexpected finding. Unfortunately, just when it appeared likely that a similar result would be the outcome, a virulent outbreak of respiratory catarrh and pneumonia due to a streptococcal infection occurred in the stables. Several of the animals in the experiment were so severely affected that the results of the virus injections were completely obscured, and a third more comprehensive experiment was carried out as indicated below. All the animals were injected on 23/6/33 and the dose in each case was 10 c.c. of the indicated dilution of brain material subcutaneously.

GROUP 1.—*Strain O Virus after 40 Passages in Mice.*

Horse.	Dilution.	0·05 c.c. in Mice Intracerebrally.	Reaction.
20541	1/100	4 : 4	Reaction from 4th day. Died 9th day.
20542	1/100	—	Reaction from 3rd day. Died 6th day.
20543	1/10,000	5 : 6	Reaction from 3rd day. Died 9th day.
20544	1/10,000	—	Reaction from 4th day. Died 9th day.

GROUP II.—*Strain O Virus after 21 Passages in Guinea-pigs.*

Horse.	Dilution.	0 05 c.c. in Mice Intracerebrally.	Reaction.
20572	1/50	5 : 6	Reaction from 3rd day. Died 9th day.
20573	1/50	—	Reaction from 3rd day. Died 10th day.
20581	1/500	5 : 7	Reaction from 4th day. Died 9th day.
20582	1/5,000	6 : 6	Reaction from 3rd day. Died 9th day.
20583	1/5,000	—	Reaction from 4th day. Died 10th day.

GROUP III.—*Strain 20449 after 51 Passages in Mice.*

Horse.	Dilution.	0 05 c.c. in Mice Intracerebrally.	Reaction.
20545	1/100	4 : 4	Mild fever from 19-23rd day. Recovered.
20546	1/100	—	No reaction.
20569	1/1000	4 : 5	,,
20570	1/10,000	5 : 6	,,
20571	1/10·000	—	,,

GROUP IV.—*Strain 20449 after 23 Passages in Guinea-pigs.*

Horse.	Dilution.	0·05 c.c. in Mice Intracerebrally.	Reaction.
20585	1/50	5 : 6	Too wild to temperature. No clinical reaction.
20586	1/50	—	No reaction.
20587	1/500	5 : 7	Moderate reaction from 8th to 11th day. Recovered.
20588	1/5,000	6 : 7	No reaction.
20589	1/5,000	—	,,

RESULTS.

Four horses received various dilutions of O virus which had been passaged for 40 generations in mice and five horses received O virus after 21 passages in guinea-pigs. All died from horsesickness as a direct result of the injection.

Five horses received various dilutions of virus, strain 20449, after 51 generations in mice, and five horses received strain 20449 after 23 generations in guinea-pigs. Of the 10 animals eight showed no clinical or febrile reaction, one was too wild to temperature but showed no clinical reaction, one reacted mildly, and one showed a fairly severe reaction but made a rapid recovery.

At various times after recovery all the survivors were shown to be solidly immune to the homologous strain of virus.

CONCLUSIONS.

After 40 generations in mice and 21 generations in guinea-pigs O virus had not reached a level of attenuation sufficient to permit of recovery of any of the injected horses. Some attenuation probably had occurred, since the course of the disease was prolonged about 3 days beyond the time that would have been expected from the use of fully virulent virus.

After a slightly greater number of generations in both mice and guinea-pigs strain 20449 appeared to have become sufficiently attenuated to render its injection into horses perfectly safe. This great difference in the results obtained with the two strains of virus cannot be ascribed solely to the slight difference in number of brain to brain passages in the small animals. Either O virus is a strain which does not lend itself to rapid attenuation or after its 193 passages in horses it had become "fixed" for horses, with the result that attenuation probably will take place eventually but will take very much longer. The latter is the more acceptable explanation, since strain 20449 was originally no less virulent for horses, but, as it was isolated in its first generation in horses, it is capable of undergoing a metamorphosis from viscerotropism to neurotropism more rapidly and more completely.

In this experiment no difference could be detected in the results obtained from the injection of the highly concentrated or diluted brain emulsion. No explanation for this discrepancy can be offered. From the point of view of ultimate economic production of a vaccine, it has not been determined what concentration of brain material will constitute the most efficient antigen, nor how many doses can be obtained from a single mouse or guinea-pig brain.

B. THE IMMUNIZATION OF MULES.

The experiments carried out on horses indicate that a gradual progressive attenuation results from the serial passage of the virus through mice and guinea-pigs by the intracerebral route. After a limited number of passages the injection of a partially fixed neurotropic virus, not unexpectedly, may produce severe reactions and even some mortality in highly susceptible horses. Since mules are considerably more resistant than horses, it was decided at this stage to attempt the immunization of a few mules in the hope that some light might be thrown on the change which was taking place in the virus.

The virus strains had been passaged for a greater number of generations in mice than in guinea-pigs so that in a preliminary experiment on 10 mules the "mouse" virus was used.

The injections given and the results obtained are shown in tabular form below. It will be noticed that the animals were divided into two groups. Group I were given an initial injection of various dilutions of brain material representing mouse generation 34, strain 20449; after completion of the immunity test against the homologous strain of this virus they received various dilutions of material representing mouse generation 35, strain O. In group II the reverse procedure was adopted. The primary injections comprised various dilutions of mouse generation 29, strain O, and after the homologous immunity test mouse generation 49, strain 20449, was given.

The second table records the infectivity of the different virulent brain emulsions, as indicated by titration in mice.

TABLE I.

Mule.	Date.	Mouse gn. 34/20449.		Result.	Immunity Test 5 c.c. Virulent blood i.v.		Mouse gn. 35/0.			Result.	Immunity Test 5 c.c. O Virus i.v.	
		Dose.	Conc.		Date.	Result.	Date.	Dose.	Conc.		Date.	Result.
20597	3/4/32	20 c.c.	1/25	No reaction	25/4/33	Fever 7th to 14th day	27/5/33	10 c.c.	1/25	No reaction	16/6/33	No reaction.
20598	3/4/33	10 c.c.	1/250	Mild fever 9th to 16th day	25/4/33	Slight fever 10th to 16th day	27/5/33	10 c.c.	1/100	No reaction	16/6/33	No reaction.
20599	3/4/33	10 c.c.	1/2,500	Severe reaction 7th to 16th day	25/4/33	Slight fever 7th to 16th day	27,5/33	10 c.c.	1/1,000	Intermittent fever 9th to 14th day	16/6/33	No reaction.
20600	3/4/33	10 c.c.	1/25,000	Slight fever 7th to 13th day	25/4 33	Slight fever 8th to 17th day	27, 5, 33	10 c.c.	1/10,000	No reaction	16/6/33	No reaction.
20601	3/4/33	10 c.c.	1/250,000	No reaction	25,/4/33	Reaction from 4th day. Died 7th day	—	—	—	—	—	—

Mule.	Mouse gn. 29, O.				Immunity Test 5 c.c. O Virus i.v.		Mouse gn. 49/20449.				Immunity Test 5 c.c. Virulent blood i.v.	
	Date.	Dose.	Conc.	Result.	Date.	Result.	Date.	Dose.	Conc.	Result.	Date.	Result.
20602	25/4/33	10 c.c.	1/25	Reacted from 10th day. Died 13th day	—	—	—	—	—	—	—	—
*20604	25/4/33	10 c.c.	1/25	Severe clinical reaction from 7th to 16th day	19/5/33	No reaction	8/6/33	10 c.c.	1/25	No clinical reaction	23/6/33	No reaction.
20605	25/4/33	10 c.c.	1/100	No reaction	19/5/33	No reaction	8/6/33	10 c.c.	1/100	No reaction	23/6/33	No reaction.
20607	25/4/33	10 c.c.	1/1,000	No reaction	19/5/33	No reaction	8/6/33	10 c.c.	1/1,000	No reaction	23/6/33	No reaction.
*20608	25/4/33	10 c.c.	1/10,000	No clinical reaction	19/5/33	No reaction	8/6/33	10 c.c.	1/10,000	No reaction	23/6/33	No reaction.

* Too wild to temperature.

† The blood used for this immunity test was obtained from a horse which contracted natural horsesickness on a farm (Kaalplaas) some 8 miles from Onderstepoort. The virus is similar to but not identical with strain 20449 and consequently the slight reactions in the mules on immunity test can be explained.

TABLE II.—*Vitrus Titration in Mice. Dose* 0·05 *Intracerebrally.*
GROUP I.

Generation 34.	Strain 20449.	Generation 35.	Strain O Virus.
Dilution.	Course in Days.	Dilution.	Course in Days.
1/25	6 : 7	1/25	6 : 7
1/250	5 : 5	1/100	6 : 7
1/2,500	5 : 6	1/1,000	5 : 5
1/25,000	5 : 7	1/10,000	6 : 7
1/250,000	8 : 0	1/100,000	0 : 0
1/2,500,000	0 : 0	1/1,000,000	0 : 0

GROUP II.—*Generation 29, Strain O Virus. Generation 49,
Strain 20449.*

Generation 29.	Strain O Virus.	Generation 49.	Strain 20449.
Dilution.	Course in Days.	Dilution.	Course in Days.
1/25	8 : 8	1/25	5 : 5
1/100	7 : 8	1/100	4 : 5
1/1,000	8 : 9	1/1,000	5 : 5
1/10,000	9 : 0	1/10,000	6 : 7
1/100,000	0 : 0	1/100,000	6 : 6
		1/1,000,000	0 : 0

RESULTS.

Group 1.—In this group the process of immunization of five
animals was commenced by giving various dilutions of strain 20449,
the 34th generation in mice being used. There was no mortality,
and with the exception of one mule which received the 1:2,500
dilution the reactions were so mild·that they might have escaped
clinical recognition had a bi-daily temperature record not been kept.
On immunity test, after an interval of 22 days, 4 of the mules
(20597-20600) showed definite but very mild reactions and recovered;
1 mule (20601) commenced to react on the 4th day after injection
and died on the 7th day. The reaction in this animal is of particular
interest.

According to the titration of infectivity in mice, 0·05 c.c. of
the 1:250,000 emulsion contained approximately 1 lethal dose of
virus for mice, since only 1 out of 2 injected mice died and death
occurred at least a day later than was anticipated. Therefore, it is
not unreasonable to assume that the 10 c.c. of this dilution injected
into the mule (20601) contained less than 1 mule-infective dose. If
less than a single infecting dose was injected there would be no
multiplication of the virus in the body and no immunity would
result. Consequently the death of the animal on immunity test is
easily explained, and this animal serves as a control for the virulence
of the blood used.

After an interval of 32 days, the four surviving mules were
injected with virulent brain emulsion comprising mouse generation
35, strain O, in dilutions varying from 1/25 to 1/10,000. There

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was a slight fever reaction in one animal (20599) which had been injected with the 1/1,000 dilution. The remainder showed no clinical or febrile reaction, and all were solidly immune 20 days later.

Group II.—Immunization of five mules was commenced with the injection of various dilutions of virulent brain material comprising generation 29, strain O. The two animals which received the highest concentration reacted severely; the one (20604) recovered and the other (20602) died, the long incubation period, however, indicating that an attenuation of the virus had taken place. The remaining three mules did not react. On immunity test after an interval of 24 days, the four surviving mules proved to have developed a solid immunity.

In the light of the experience with mule 20601 in group I above, the failure of mule 20608 to react to the immunizing injection, and yet proving to be immune subsequently is of interest. According to the titration of infectivity in mice the 1/10,000 dilution used contained barely one mouse-infecting dose, since only one out of two injected mice died. The 10 c.c. given to the mules, therefore, must have contained only slightly more than 1 mule-infective dose and consequently an active immunity developed. It is unfortunate that the animal was too vicious to temperature, because the previous results have shown that often it is necessary to differentiate between a clinical and merely a febrile reaction.

In addition, it is worthy of note that the animals which reacted very severely both received the higest concentration of brain material containing a virus which had been subjected to a limited number of passages—29. This result is precisely the opposite to that obtained from the use of guinea-pig brain emulsion in horses. This observation is discussed later.

After an interval of 20 days the four mules received various dilutions of brain emulsion comprising generation 49, strain 20449. There were no reactions and 15 days later a solid immunity to the homologous strain of virus had developed.

It is difficult to put forward a convincing comparison of the results obtained in group I and group II, since adequate data have not been collected in regard to the degree of cross-immunity existing between the two strains of virus used before neurotropic fixation. But, it is interesting to note that immunization against one strain produced an undoubted immunity against the other, since the first series of immunizing injections produced reactions which were considerably more severe than those resulting from the second virus. This difference cannot be ascribed entirely to the viruses used being at different levels of attenuation by passage. Further work is in progress on the antigenic structure of the different strains of virus isolated so that a full discussion on cross-immunity will have to be reserved for a later publication.

CONCLUSIONS.

The results obtained with mules were approximately those anticipated from a consideration of the previous results obtained with horses. It is shown to be possible to immunize mules by the injection of a modified virus which has been attenuated by serial passage

through both mice and guinea-pigs. Moreover, every animal which survives an injection of a certain infective dose of neurotropic virus, irrespective of whether a demonstrable reaction or not is produced, is immune to the homologous strain as contained in virulent equine blood. The more resistant species, the mule, is able to withstand the injection of a partially fixed neurotropic virus better than horses, and it is shown that the one strain of virus (20449) is attenuated at a greater speed than the other strain (O).

The results were so encouraging that it was decided to run a second experiment on 10 mules with the object of—

(1) comparing the relative value of the guinea-pig and mouse viruses;

(2) comparing the production of immunity as a result of the injection of minimal doses of infective emulsion;

(3) ascertaining whether a series of injections of different strains of virus could be replaced by a single injection of a bivalent mixture, i.e. to obtain some information as to the possibility of producing a polyvalent vaccine consisting of a mixture of known antigenically different strains.

The injections given and the results obtained are shown in tabular form below. It should be stated that the different dilutions of each strain from both guinea-pigs and mice were made up separately, and after dilution the requisite mixtures were made.

A. Guinea-pig Virus.—Generation 23, Strain 20449 + Generation 21, Strain O.

Mule.	Date.	Dose.	Dilution.	Result.
20596	23/6/33	10 c.c.	1/50	Moderate reaction from 4th to 12th day.
*20603	23/6/33	1/50	1/50	Very mild indefinite febrile reaction.
20606	23/6/33	10 c.c.	1/500	Moderate reaction from 4–14th day.
20610	23/6/33	10 c.c.	1/5,000	Severe reaction from 4–16th day. Recovered.
20609	23/6/33	10 c.c.	1/5,000	Mild febrile reaction from 7–14th day.

B. Mouse Virus.—Generation 51, Strain 20449 + Generation 40, Strain O.

Mule.	Date.	Dose.	Dilution.	Result.
20611	23/6/33	10 c.c.	1/100	Reaction commenced 3rd day. Died 11th day.
20612	23/6/33	10 c.c.	1/100	Severe reaction from 4–16th day. Recovered.
20613	23/6/33	10 c.c.	1/1,000	Reaction commenced 4th day. Died 12th day.
*20614	23/6/33	10 c.c.	1/10,000	Mild indefinite febrile reaction.
20615	23/6/33	10 c.c.	1/10,000	Moderate reaction from 5th to 11th day.

* NOTE.—After an interval of 28 days, mule 20603 received an immunity test of 5 c.c. of virulent blood strain 20449 intravenously, and mule 20614 5 c.c. of virulent blood strain O. neither animal showed any reaction so the remainder were turned out to grass to be exposed to natural infection.

Results.

Of the five mules which received dilutions of the guinea-pig virus all showed definite reactions, but only one (20610) reacted so severely that any doubt was entertained as to its ultimate recovery.

In group B, which received mouse virus, all the mules reacted. The reactions clinically were of a much more severe nature than in group A, and there were two deaths.

The immunity test which was carried out may be considered inadequate, but it must be borne in mind that previous work has shown conclusively that every animal which receives a sure infecting dose of virus develops a solid immunity. Hence the demonstration that immunity against both strains of virus had been induced was regarded as sufficient. During the period the mules have been at grass there has been no natural outbreak of horsesickness, so that no additional information has been collected.

Conclusions.

Again it is demonstrated that horsesickness virus is more rapidly attenuated by passage through the guinea-pig than through the mouse, but it cannot be stated whether the attenuation after complete fixation in both animals eventually would attain the same level. This point will be tested adequately in due course.

It is shown that it is possible to immunize against two strains of virus simultaneously, but when two partially attenuated strains are used the reactions produced by either strain separately are considerably less severe than the reactions produced by the injection of a mixture of equal parts of the two strains.

Attention must be directed to the fact that the two deaths and possibly the most severe reaction occurred in those animals which received the highest concentration of mouse virus, and the mildest reactions occurred in those animals which received the highest concentration of guinea-pig brain emulsion. The possible significance of this finding is discussed below.

Lastly it is clear that high dilutions of virulent brain emulsion make satisfactory antigens so long as one or more minimal infective does are injected. This point is of extreme importance from the point of view of the ultimate economic production of a vaccine in bulk.

DISCUSSION.

The experiments on mules and horses detailed above indicate conclusively that there is a profound alteration in the nature of the virus of horsesickness as a result of serial brain to brain passage through both mice and guinea-pigs. No accurate data as to the virus content of virulent equine blood have been collected, since the cost of carrying out an accurate titration in horses is prohibitive; but the alteration in the virus on neurotropic fixation represents not a decrease in infectivity, since high dilutions of brain emulsion are infective, but does represent some change in the actual nature of the virus resulting in a marked natural attenuation. The change from viscerotropism to neurotropism is not accompanied by any apparent alteration in antigenic power. Consequently the use of a neurotropic virus " fixed " for mice or guinea-pigs has been shown to constitute a rational method of immunization against horsesickness.

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Attenuation by passage through the guinea-pig occurs at a greater rate than by passage through the mouse, but no opinion can be expressed as to the ultimate level which will be attained after repeated subinoculation over a number of years. Whether the degree of attenuation in either animal will eventually reach such a point that injection into horses will be perfectly safe in every case, has yet to be determined. This point will only be cleared up by the collection of data from a large number of animals since the susceptibility of individual horses is known to vary within wide limits. At the present moment it is hoped that attenuation will reach a level which is consistently safe and that it will not be necessary to resort to the preliminary injection of a virus artificially attenuated by chemical or physical means, or to modify the reaction by the simultaneous injection of hyperimmune serum.

A consideration of the results obtained from the experiments recorded indicates that when once the anti-body producing mechanism of the animal body has been stimulated, it is comparatively easy to reinforce the initial immunity. This point was demonstrated by the mildness of the reactions produced by neurotropic virus O in animals previously immunized against strain 20449, whereas the same material produced severe reactions and even mortality in fully susceptible animals. Consequently, it has still to be decided whether the ultimate procedure will consist of a series of injections of different virus strains or a single injection of a polyvalent mixture. The possibility of the latter procedure has been demonstrated by the successful immunization of mules against two viruses by a single subcutaneous injection.

This conception of the production of a polyvalent vaccine constitutes a considerable advance over previous methods. Both the hyperimmune serum-virus simultaneous method and the formalized spleen virus method have as their basis the use of a stingle strain (strain O) which, experience has shown, is capable of immunizing against a greater number of different strains than any other virus isolated. Yet the immunity produced by these methods is not complete as evidenced by periodical breakdowns in the field; practical difficulties and cost prevented any attempt at producing either a polyvalent serum or formalized vaccine. With the neutrotropic virus method an attempt certainly can be made to immunize against all strains that are isolated, since a technique of *in vitro* neutralization* has been developed which permits of an evaluation of the antigenic structure of the different natural strains of virus which have been encountered.

In conclusion it must be pointed out that up to the present it has merely been shown that immunization by means of an attenuated neurotropic virus is possible. The work on mass production of such a vaccine is still in its initial stage, since a very large number of important points have yet to be subjected to a thorough investigation. For example, the relative efficacy of the mouse and guinea-pig adapted viruses have not been evaluated. The ease of serial transfer and the short course of the disease produced in mice gives preference to this animal for rapid passage and maintenance of a virus. On the other hand, the guinea-pig possesses a brain which is approximately 5 times the size of that of the mouse, though the

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* This forms the basis of a report to be published later.

barins of moribund mice have been found to contain about 10 times more virus per unit of brain substance so that again preference must be given to the mouse for the production of large qualities of virus. In spite of this, attention must be directed to the phenomenon of high concentrations of virulent guinea-pig brain on some occasions producing very much milder reactions than low dilutions, whereas the reverse has been the experience with mouse material. No adequate explanation can be offered for the phenomenon at this stage, but the tentative suggestion is advanced that the prolonged development of the virus within the cells of the guinea-pig brain may result in the production of a non-infective antigenic substance in appreciable amount analogous to the specific poly-saccharide antigens of some bacteria.

This report therefore must be regarded as being of the nature of a progress report since, to be of any real value, work on the immunization of equines against horsesickness must be carried out on large numbers of animals because the individual susceptibility of different horses varies so greatly that a true interpretation of the relative value of different methods of immunization is only possible after the collection of adequate data from work on large numbers of animals housed and maintained under a variety of conditions.

SUMMARY.

(1) Details of the results obtained from the injection of horses and mules with neurotropic mouse and guinea pig adapted virus are given.

(2) It is shown that the virulence of horsesickness virus progressively decreases for equines as neurotropic fixation takes place by serial passage through mice and guinea-pigs.

(3) The attenuation occurs more rapidly through the guinea-pig, but it is not known whether the ultimate level will not be the same.

(4) All animals which survive an injection of one infective dose of neurotropic virus, whether or not a demonstrable reaction is produced, are immune to the homologous strain of virus. Immunity to heterologous strains is at most only partial.

(5) No difference in favour of either the subcutaneous or intravenous method of injection could be determined.

(6) It is shown that the subcutaneous injection of as small a dose as 10 c.c. of a 1:10,000 dilution of infective brain emulsion is adequate.

(7) Attention is directed to the phenomenon of a high concentration of infective guinea-pig brain emulsion producing a milder reaction than a low but still infective concentration.

(8) The possibility of developing a polyvalent vaccine is discussed

REFERENCES.

NIESCHULZ, O. (1932). Over die infectie van muizen met het virus der Zuid-Afrikaansche paardenziekte. *Tydsch. voor Diergeneesk.* Vol. 59, No. 24, pp. 1433-1445.

ALEXANDER, R. A. (1933). Preliminary notes on the infection of white mice and guinea pigs with the virus of horsesickness. *Jnl. S.A.V.M.A.*, Vol. 4, No. 1, pp. 1-9.

SAWYER, W. A., KITCHEN, S. E., & LLOYD, W. (1932). Vaccination against yellow fever with immune serum and virus fixed for mice. *Jnl. of Exp. Med.*, Vol. 55, No. 6, pp. 945-969.

The Occurrence and Identification of Blue-tongue in Cattle—the so-called Pseudo-Foot and Mouth Disease in South Africa.

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INTRODUCTION.

Up to the present (1932) it has been accepted that bluetongue (Catarrhal fever) is a disease specifically and exclusively confined to sheep. No observations have been recorded of the disease occurring naturally in cattle, and attempts to produce reactions in animals other than sheep, have generally failed.

Spreull (1905) demonstrated that calves and goats inoculated with blood of sheep affected with bluetongue retained the virus for some time. He found the blood of such calves still infective when tested on sheep on the 21st day after inoculation, but after 35 days the blood was found to be sterile. Subsequently he again inoculated these calves with virus material, but says: '' The calves did not again become virulent ''. Apart from a '' slight reaction '' in one of these calves he apparently did not observe any typical bluetongue symptoms or lesions. The fact, however, that the blood of these calves did not become virulent, when inoculated a second time, would seem to indicate that the calves had undergone a reaction. The reaction was, however, imperceptible as no symptoms or lesions were observed. When the virus was introduced in the second instance it was apparently destroyed by immune bodies which had developed after the first inoculation.

More recently Neitz (1933) found that the blesbuck (*Damaliscus albifrons*), although developing no perceptible reaction to bluetongue virus, can in a similar way act as a '' reservoir '' for this virus. He could demonstrate the presence of the virus in the blood of the blesbuck up to the 17th day after inoculation (a period corresponding closely to Spreull's 21 days in the case of calves). Neitz puts forward

the interesting hypothesis that the disease bluetongue was originally transmitted from game to sheep: " The game acting as a reservoir for this virus in the same way as the blue and black wildebeeste act as a reservoir for Snotsiekte ". This contention, however, appears to be somewhat misleading, and the analogy made between bluetongue and snotsiekte is not strictly correct. In the case of snotsiekte outbreaks have only been noted where cattle come into very close contact with wildebeeste (Mettam, 1923). Furthermore, under natural conditions there is no evidence of the disease spreading from cattle thus infected. On the available evidence the wildebeeste do seem to serve as a reservoir for snotsiekte virus. In the case of bluetongue, however, the presence of game is not a requisite factor and the epizootology of the disease is entirely different from that of snotsiekte. Therefore, the suggestion that game should be inculpated as the immediate reservoir of bluetongue virus cannot be entertained.

The intensive veterinary campaign of 1932-33 to combat the outbreak of foot and mouth disease in Southern Africa brought to light the occurrence of an undescribed " stomatitic " disease of cattle which made its appearance in localities scattered over an extensive area of the Union (vide Appendix C). In practically every instance where the disease was reported the owners suspected foot and mouth disease and widespread consternation resulted. These outbreaks were particularly common on Transvaal and Free State highveld farms.

At the beginning of March, 1933, the disease broke out in a herd on the farm Welgezegend in the Standerton District. Three cows developed very severe symptoms. In this instance also the owner suspected foot and mouth disease, and as the farm was not very far from Rooikop No. 15 (near Germiston), where foot and mouth disease had been diagnosed, the probability of another outbreak seemed very real.

Transmission experiments were decided upon and carried out on this farm. These experiments were in the first place designed to exclude foot and mouth disease and, in the event of its exclusion to attempt to ascertain the nature of this new condition.

The results of these Welgezegend experiments and of subsequent investigations undertaken at Onderstepoort, are the subject of this paper.

With the available experimental data it would appear that the virus causing the well-known bluetongue of sheep is also the factor concerned in the aetiology of this stomatitic disease of cattle.

For the sake of convenience and to prevent misunderstanding, it is proposed to refer to the disease as *Bluetongue of cattle*. It should, however, be understood that, although the term bluetongue is now almost exclusively used to designate this disease in sheep, it is an undesirable nomenclature when applied to the disease in cattle, since the blue or cyanotic condition of the tongue, as observed in sheep, is not usually manifested. A very regular change is a localized inflammation with necrosis of the buccal and nasal mucosa

and a popular term such as "seerbek" or "sore mouth" would have been more appropriate. By the adoption of the name *Bluetongue in cattle*, it is, therfore, not intended to refer to pathological or symptomatological changes in this disease, but rather to the aetiological factor concerned.

HISTORICAL.

Keppel and Robinson (1932) report on an outbreak of an "*Ulcerative stomatitis*" in cattle on several farms in the Eastern Free State and Basutoland. From their description of this disease there appears to be no doubt that they were also dealing with bluetongue in cattle. Keppel and Robinson suggested that the symptoms are to some extent reminiscent of three-day-stiffsickness. They undertook transmission experiments on a very limited scale, but failed to reproduce the condition. According to them, F. A. Verney, the Principal Veterinary Officer of Basutoland, encountered the disease at least 17 years ago. From evidence gathered from farmers there is reason to believe that the disease is not new to South Africa.

No serious notice was taken of the condition, for usually only a single bovine or at the most a few become affected, and such animals usually recovered. It was when a differential diagnosis became imperative that it received the serious attention of the Veterinary Research Division.

The term "*Ulcerative stomatitis*" and the less appropriate one of "*Pseudo-Foot and Mouth disease*", have been used to describe the condition. Farmers in the Free State now refer to the disease as "*seerbek*" (i.e. sore mouth).

Various theories and suggestions have been advanced by veterinarians and farmers as possible explanations for the cause of the disease. Keppel and Robinson (*loc. cit.*) mention that the stomatitis, described by them, might be a symptom of a general septicaemia such as may be caused by a filterable virus. It has been put forward that a poisonous plant might be responsible, but in view of the occurrence of the disease over a very wide area (*vide* Appendix C), and in localities differing markedly in their floristic constitution, this suggestion may be dismissed. Chemical irritants, such as caustic soda, carbolic acid preparations, etc., were also suggested, but this must also be ruled out for in many instances there was no history of the presence of such chemicals. A specific bacterial infection has been thought of. In cases where there was a lingual protrusion the disease was confused with lamsiekte (bovine botulism).

One of the authors (J. G. B.) whilst investigating some of these cases in the Standerton area, expressed the opinion that this disease was similar to, if not identical with, bluetongue in sheep.

CLASS OF ANIMALS AFFECTED.

It would appear from the cases which come under observation that all breeds of cattle are susceptible. The disease was observed in the Friesland, Afrikander, Shorthorn and Hereford breeds. Age

is apparently not an important factor as animals of varying ages contracted the disease. It is noteworthy, however, that the disease was not encountered in very young calves. In all the cases which came under observation the disease occurred in cattle pastured on the open veld. It was not seen in cattle which were housed. The absence of the disease in calves can probably be accounted for by the fact that, under the fairly intensive farming conditions of the Transvaal and Free State areas, where this disease was particularly prevalent, the calves are usually kept in kraals or stables, particularly at night, and are not allowed to run with their mothers. In this way they probably escaped the dangers of natural infection. The possibility, however, that young calves may be relatively less susceptible to the disease than older ones might also account for it's infrequent appearance in very young stock.

EPIZOOTOLOGY.

As has already been mentioned, this disease was observed over a wide area of the Union during 1933. According to du Toit (1933) reports of a similar disease have been received from territories outside of the Union, e.g. Northern Rhodesia.

During 1933 the outbreaks were encountered during the late summer and autumn, and most cases were reported during March and April. Keppel and Robinson (*loc. sit.*) also encountered an " *Ulcerative stomatitis* " during the same time in 1932. As far as it known no outbreaks have been noticed during the winter months. Just as in the case of bluetongue in sheep, further outbreaks are probably arrested by the first frosts. A fairly constant feature of the disease in cattle would seem to be that a comparatively small number (about 0·5 to 2 per cent.) in a herd become affected, but according to a communication received from J. J. Keppel, Senior Veterinary Officer of the Free State, the disease may assume alarming proportions. He mentions an outbreak where practically all the animals in a herd became affected.

There is no evidence to show that the disease can spread by contact. Even in instances where diseased cows were confined with their calves in a small stable and where the calves suckled or attempted to suckle the sore teats, the disease did not spread.

As in the case of bluetongue of sheep and horsesickness the outbreaks occur sporadically. It should be mentioned that during the 1933 season, when so many outbreaks of this disease in cattle were encountered, horsesickness and bluetongue in sheep were prevalent over a very large area of South Africa and even occurred in areas where it was practically unknown before. These two diseases actually occurred simultaneously with the disease in cattle on some of the farms where the outbreaks were investigated.

The same epizootological factors concerned in the occurrence of bluetongue of sheep and horsesickness are apparently also operative in this disease in cattle. It is, of course, well known that horsesickness and bluetongue do not always occur with the same severity, nor are the diseases as widespread in some years as in others. By analogy it can be inferred that this disease in cattle

does not occur to the same extent every year, in fact during some years it may actually not even make its appearance. The telluric conditions during 1933 were probably particularly favourable for its occurrence and apparently the same was the case 17 years ago when F. A. Verney (*vide* Appendix C) observed a widespread outbreak of an apparently similar condition in Basutoland.

AETIOLOGY.

Transmission experiments were undertaken with blood from twelve cases of this cattle disease in eight different outbreaks on the following farms, some of them very widely situated from one another: *Welgezegend, Kromdraai, Darling* and *Elandslaagte* (all in the Standerton District), *Novo* (Wepener), *Onlangs* (Frankfort), *Montague* (Smithfield) and *Swartland* (Northern Potgietersrust).

The presence of the virus in the blood of the affected cattle was ascertained by inoculating normal sheep and noting the reactions which followed. Investigations were also undertaken to determine whether calves were susceptible to the virus. The results of these transmission experiments are fully described in Appendix A and are summarized in the following table:—

<div align="center">SUMMARY OF TRANSMISSION EXPERIMENTS.</div>

Serial Number of Experiment.	Source of virus.	Normal sheep.			Normal calves.	
		No. inoculated.	Result.		No. inoculated.	Result: No. of reactors.
			No. of reactors.	No. of deaths.		
1 (a)	Welgezegend—Cattle—Cows...	15([1])	15	9	5	4
1 (b)	Welgezegend—Cattle—Cows...	6	6	4	16([2])	12
1 (c)	Welgezegend—Cattle—Ox.....	16	16	6	3	3
2	Kromdraai—Cattle...........	14	14([3])	8	2([4])	2
3	Darling—Cattle..............	4	4	2	—	—
4	Elandslaagte—Cattle.........	2	2	1	—	—
5	Novo—Cattle................	4	4	1	—	—
6	Montague—Cattle............	2	2	0	—	—
7 (a)	Onlangs—Cattle (Case I)......	6	0	0	—	—
7 (b)	Onlangs—Cattle (Case II).....	4	4	0	—	—
8 (a)	Swartland—Cattle (Case I)....	2	0	0	—	—
8 (b)	Swartland—Cattle (Case II)...	4	0	0	—	—
9	Novo—Sheep................	18	18([5])	3	—	—

NOTE.—(1) Does not include two sheep which received an intranasal injection of virulent blood and developed no reaction.

(2) Includes five calves which were injected intranasally with a mixture of milk, urine, emulsified necrotic tissue and blood from three affected cows. Four out of these five calves developed definite reactions.

(3) Includes one doubtful reaction.

(4) *Vide* experiment 10 (*d*).

(5) Does not include sheep which reacted to cattle strains and where a slight reaction again developed to this sheep strain, e.g. one animal in experiment 1 (*a*) (*vide* table of the Summary of the Immunity Tests).

In the outbreak at Swartland [i.e. experiments 8 (*a*) and 8 (*b*)] subinoculations failed to demonstrate the presence of an incitant in the blood of two affected cases. It should be mentioned that blood was collected from these animals in the final stages of the disease and the avirulence of the blood could probably be attributed to this. Even in field outbreaks of bluetongue in sheep, where there is little doubt about the diagnosis of the disease, it is not always possible to recover the virus from every case.

It would seem advisable to collect blood for experimental purposes during the initial stages of the disease, there being more certainty of recovering the virus in this way.

In the outbreak at Onlangs, blood samples were taken from two cases. The blood from one animal (an advanced case) was found to be inactive; but that from the other case, which showed the disease in the initial stages at the time when the blood sample was collected, proved to be virulent.

The immunity of a number of sheep which had recovered from very severe reactions produced by the infective material contained in the blood of cattle affected with this disease, was tested against a virulent bluetongue virus collected from sheep on the farm Novo in Wepener district. The virulence of this sample of blood was thoroughly tested (*vide* experiment 9). The susceptibility of sheep which had been inoculated with and had reacted to Onderstepoort bluetongue vaccine strain was also determined. (Virulent bluetongue virus becomes attenuated by continuous passage through a number of generations in sheep, and it is this attenuated virus which is used as a vaccine. The reactions provoked by the vaccine are of an abortive nature, i.e. a rise in body temperature occurs after a more or less definite incubation period, and in very rare cases a slight hyperaemia of the buccal mucosa is noted.)

The results of these immunity tests are summarized in the following table:—

SUMMARY OF IMMUNITY TEST.

Serial Number of experiments.	A.—Tests in sheep which recovered from reactions produced by various field strains.				B.—Tests in sheep which recovered from bluetongue vaccine reactions.		
	Strain from which sheep recovered.	Immunity tested with.	No. inoculated.	Result: No. which reacted.	Immunity tested with strain from.	No. inoculated.	Result: No. which reacted.
1 (a)	Welgezegend cattle (ex. cows)	Novo sheep strain (natural B. T. outbreak)	4	1	Welgezegend cattle (ex. cows)	4	0
1 (c)	Welgezegend cattle (ex. ox)	,, ,,	2	0	Welgezegend (ex. ox)	5	0
2	Kromdraai cattle.	,, ,,	2	0	Kromdraai cattle	5	0
3	Darling cattle....	,, ,,	2	0	Darling cattle..	5	0
4	Elandslaagte cattle	,, ,,	1	0	—	—	—
5	Novo cattle......	,, ,,	2	0	—	—	—
6	Montague cattle..	,, ,,	2	0	—	—	—
7 (a)	Onlangs—Neg. reactors (ex. Case I)	,, ,,	5	5	—	—	—
7 (b)	Onlangs cattle (ex. Case II)	,, ,,	4	1(²)	Onlangs cattle.	5	0
8 (a)	Swartland — Neg. reactors (ex. Case I)	Welgezegend cattle	2	2	—	—	—
8 (b)	Swartland — Neg. reactors (ex. Case II)	,, ,,	2	2	—	—	—
		Novo sheep virus	2	2			
9	Novo sheep......	Welgezegend cattle	1	0	Novo sheep....	5	2(³)
		Kromdraai cattle.	2	0			

NOTE.—(1) A very doubtful reaction.

       (2) A very doubtful reaction.

       (3) The reaction in one of these two sheep was very doubtful and consisted only of a rise in temperature. In the other case it was of a very mild nature, i.e. rise in temperature and slight injection of the buccal mucosa.

It is clear that :

(1) Sheep which had reacted to cattle strains of this infective material were immune to virulent bluetongue virus obtained from a sheep. Only one breakdown [experiment 1 (*a*)] occurred.

(2) Sheep immunized with bluetongue vaccine were resistant to experimental infection with strains of virus obtained from natural outbreaks in this cattle disease and from a field outbreak of bluetongue in sheep. Only three breakdowns occurred in the 29 tested animals and of these two reactions were very doubtful.

Incidentally these tests again illustrated the high efficacy of the Onderstepoort bluetongue vaccine. It would also appear that as far as the immunology is concerned no very marked difference occurs in the various field strains, as is, for instance, encountered in strains of the closely analogous disease horsesickness. Where reactions occurred in the immunity tests they were of a very mild nature in comparison with those in susceptible sheep.

(3) Sheep which had reacted to the virulent Novo sheep strain of bluetongue (experiment 9), were found to be immune to cattle strains.

(4) In experiments 7 (*a*), 8 (*a*) and 8 (*b*) where no reactions were observed with apparently avirulent cattle blood no immunity resulted.

From the results of these investigations the following inferences are made : —

I. *That the infective agent present in the blood of affected cattle in various outbreaks of this " stomatitic " disease is the same as the virus which causes bluetongue in sheep, for—*

(*a*) the very characteristic syndrome of bluetongue was observed to develop in sheep which were experimentally infected with strains collected from different outbreaks;

(*b*) *an actual immunity was produced which was found to be specific :* —

(i) Bluetongue vaccinated sheep were found to be immune to various strains; and

(ii) susceptible sheep, which recovered from the reactions produced by virus obtained from cattle, were found to be resistant to a tested virulent strain of bluetongue virus recovered from a sheep in a natural outbreak.

(*c*) The *in-vitro* characteristics of the various cattle strains are like those known and described for bluetongue virus recovered from sheep [*vide* du Toit (1929), Theiler (*loc. cit.*)], e.g. they remained active in—

(i) *O.C.G. mixture* (approximately in the following proportion: blood 50 per cent., glycerine 25 per cent., water 25 per cent., potassium oxalate ¼ per cent., and carbolic acid ¼ per cent.).

(ii) In a mixture of 50 per cent. blood and 50 per cent. glycerine.

(iii) In decomposed blood.

II. *That this virus is the cause of this cattle disease*, since —

    (*a*) *the presence of the virus* could be demonstrated in this diseased condition of cattle where,

        (i) the *lesions*, especially those noted in the buccal and nasal cavities were similar to those observed in bluetongue of sheep, and

        (ii) *histological* examination revealed similar morbid changes in tissues of analogous organs in cattle and in sheep.

    (*b*) *The epizootology of the disease* is similar in both cattle and sheep.

    (*c*) *The susceptibility* of cattle was proved in transmission experiments.

Calves inoculated with virus containing blood developed a perceptible reaction and the virus was again recovered from them. A syndrome was observed in these calves which could be closely correlated with that seen in natural cases of this disease in cattle and of bluetongue in sheep. In one case at least (calf 5263) an exact reproduction of the changes in the mouth and nose and on the muzzle as seen in typical natural cases of the disease in cattle was clearly observed; but the reaction was of a comparatively mild nature.

Although the experiments clearly indicate the susceptibility of cattle to this virus, it can also be concluded that bovines are generally far more resistant than sheep, since the reactions produced in the experimental calves were not nearly as severe as in the sheep. It would appear that only a comparatively small number of bovines are peculiarly susceptible and when such an individual becomes infected alarming symptoms manifest themselves. The fact that the disease has hitherto been observed in more or less isolated outbreaks and, furthermore, that only a few individuals in such a herd usually become affected supports this contention.

The supposition that the cattle which were suffering from this stomatitic disease could have been mere carriers of bluetongue virus and that this agent may not be the real cause of the disease, may be dismissed for the following reasons: —

    (*a*) From a consideration of the facts enumerated under (1) and (2) above.

(b) As far as is known, this is the first time that bluetongue virus has been recovered from blood of veld (field) cattle. In this connection it may be mentioned that in numerous investigations, for instance, in heartwater, many samples of bovine blood collected in the field during all seasons of the year, have been inoculated into sheep and no such cattle carriers of bluetongue virus have ever been discovered.

The sheep used in the Welgezegend experiments were obtained from a flock on a neighbouring farm. They appeared healthy when introduced into the experiments. The possibility, however, that some of these sheep might have been in the incubation stage of a natural bluetongue infection could not be overlooked. The chance of such accidental contamination was excluded by keeping a group of uninoculated sheep in contact with the experimentally infected animals and by repeating some of the tests with original material on sheep at Onderstepoort. Confirmatory results were obtained with other strains where the experimental work was entirely carried out under laboratory conditions at Onderstepoort.

The investigations were designed with the express purpose of establishing the cause of this cattle disease, but at the same time interpreting additional information in connection with certain aspects of bluetongue were obtained and it will not be irrelevant to mention some of these details here:—

(a) *The Pathogenicity of the Virus in Blood Samples.*

It will be observed when studying the mortality of the sheep in the various generations of the different strains that in most instances the virus proved to be a particularly virulent right from the first generation, and a large percentage of infected sheep died. In others, e.g. *Kromdraai strain*, the virus appeared to be less virulent when the results of the first generation are considered, but in subsequent ones it proved to be very potent. It is significant that in these cases where such mild reactions were observed, the blood samples were collected from the cases at a fairly late stage of the disease. It would seem as if the pathogenicity of the virus present in samples collected from individuals in the recovering stage of the disease is probably considerably modified by immune bodies already present in the animals. This is borne out by studying the results of *Experiment* 10.

In this experiment it will be noticed that severe reactions developed in the first group of 5 sheep and 2 animals actually died. The blood taken on the 14th day after infection from two of the recovering animals in this group set up distinct reactions in 4 sheep, but none died. On the 28th day after infection a further 4 animals were inoculated and now only two developed very mild reactions whilst the other two did not react at all. However, the latter two reacted severely when inoculated with blood collected from the original donors on the 9th day after infection. The reactions were at their height on that day in those animals. On the 42nd day blood was again taken from the two sheep and no reactions could be observed in 4 inoculated sheep.

This experiment clearly indicates that in these two cases the blood became sterile within a period of less than 42 days and that the pathogenicity of the virus contained in the blood of the recovering animals gradually became modified probably by the immune bodies which appeared in the blood of the animals.

(b) *The Presence of the Virus in Foetal Material* [vide *Experiment 10* (b)].

Two pregnant ewes used in experiment 2 died from the reactions and material was collected aseptically from the contained foetuses. In each case two sheep were inoculated. One foetus was apparently sterile, but definite reactions were produced with the blood of the second foetus. On the first occasion 1 c.c. of this foetal blood was inoculated into two sheep and one developed a peracute reaction and died, but no reaction developed in the other animal. However, when 5 c.c. of the same foetal blood was injected a very severe reaction was produced.

It would seem as if the virus was not present in any great concentration in this foetal blood and 1 c.c. was apparently the minimum infective dose.

(c) *The Disease can be Transmitted by Means of an Intranasal Injection of Virus-containing Material.*

In the early experiments at Welgezegend 5 calves received an intranasal injection of suspected material and four developed definite reactions. The virus was found to be present in the blood from one of the calves [vide experiment 1 (b)]. Subsequently [vide experiment 10 (d)] it was found that sheep could also be infected in this way.

Investigations on the physical and chemical properties of the virus obtained from cattle are at present in progress and these are being compared with those of known sheep bluetongue strains.

Addendum by R. A. Alexander.

Transmission of the Virus to Mice, Rats and Guinea-pigs.

To facilitate research into the biological properties of virus an attempt was made to establish the virus in mice, rats and guinea-pigs in the following manner:—

(a) Mice by the intracerebral and intraperitoneal routes.

(b) Rats by the intracerebral, intraperitoneal and sub-cutaneous routes.

(c) Guinea-pigs by the intracerebral, intraperitoneal and plantar pad routes.

As the source of virus, use was made of virulent blood, a virulent emulsion of the early stomatitic lesions of cases of " pseudo-foot and mouth " in sheep, as well as virulent blood of a known laboratory strain of Bluetongue of sheep.

The results were uniformly negative so that it may be concluded that so far all attempts to infect mice, rats and guinea-pigs with the strains of the two viruses available have been unsuccessful.

THE SYMPTOMATOLOGY.

(a) As Observed in Cattle in Natural Outbreaks.

According to the course and the development of the disease the cases may be considered as: (1) *typical* and (2) *atypical*.

(1) *Typical Cases.*

The usual history presented by the owners.—The affected cattle are noticed to stop grazing and do not move about with the rest of the herd. Marked stiffness and lameness are observed. At this stage the disease is not regarded seriously. The owners suspect either *three-day-stiffsickness* or a vague diagnosis of *gallsickness* it attempted. About two or three days later, however, slight frothing from the mouth and even salivation is seen and in quite a number of cases the tongues protrude prominently. It is at this stage that attention is directed to the condition of the mouth and redness and soreness of the buccal mucosa is discovered. Foot and mouth disease is immediately suspected and the presence of the condition reported as such.

For convenience the symptoms will be described under various headings.

Habitus.—In most cases the animals are to be found lying down and those still standing show little inclination to move. When forced to move the sick cattle are noticed to be distinctly lame and stiff. No attempt is made to feed and rumination is in abeyance. Frequently chewing movements and grinding of the teeth is observed.

There is a rapid loss of condition, and in lactating cows a marked reduction in milk yield takes place. Sometimes this loss in milk is the first symptom of malaise noted by the owners.

The temperature.—In the early stages a high temperature is usually present. The temperature apparently soon becomes normal or only slightly elevated in the stage when the mouth lesions are well developed (this is also the case in sheep). It is at this stage that field cases are usually reported, and the temperature is therefore not of much value for diagnostic purposes. .

The symptoms and lesions in the buccal and nasal cavities.— These are most important, and it is proposed to describe them in detail.

Frothing at the mouth is frequently evident and becomes pronounced after manipulation for the purpose of examination (*vide* Figs. 1 and 2).

Sometimes salivation may be noted and this is especially marked in cases where the tongues protrude.

The lesions in the buccal and nasal cavities are essentially those which are due to a hyperaemia of the mucosa and a localized inflammation with necrosis of the mucous membrane.

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In comparatively *mild cases* the unpigmented portions of the mucosa are reddened and superficial lesions of a localized stomatitis with necrosis are to be found on the dental pad, borders of the lips, ventral aspect of the apical portion of the tongue, etc. These lesions are not very well defined, and their borders gradually merge through a hyperaemic zone into the normal mucosa. The lesions present the following appearance—the epithelium has a yellowish colour and is usually still partially attached to the underlying tissues giving an impression of a membranous deposit. Where the epithelium is detached the underlying tissues are reddened and bleed easily.

FIG. 1.

Onlangs Case: Note frothing and salivation and dried mucus on nostrils, etc., and catarrh of eyes.

In the *more severely affected* cases these lesions are more extensive. The lips, muzzle, external nares, tongue, dental pad, conical papillae of the cheeks, etc., are all involved. A marked reddening of the unpigmented portions of the buccal mucosa is seen, this is particularly marked where actual lesions occur. On pigmented areas the necrosis presents a dirty greyish appearance.

At this stage there is usually a fairly copious nasal discharge which may be catarrhal, muco-purulent and even muco-haemorrhagic. Presumably on account of the painful condition of the tongue and mouth little attempt is made to clean the nostrils with the result that the nasal discharge dries on the surfaces of the external nares (*vide* Fig. 3).

FIG. 2.
Onlangs Case: Note frothing at the mouth and incrustations on muzzle.

FIG. 3.
Onlangs Case: Note extensive excoriations and incrustations on muzzle, lips, and dried nasal discharge on external nares.

The lips are swollen and the skin of lightly pigmented areas reddened. Necrotic lesions develop along their borders and on inner surfaces. These lesions are particularly evident on the areas immediately opposite the incisor teeth (*vide* Fig. 4).
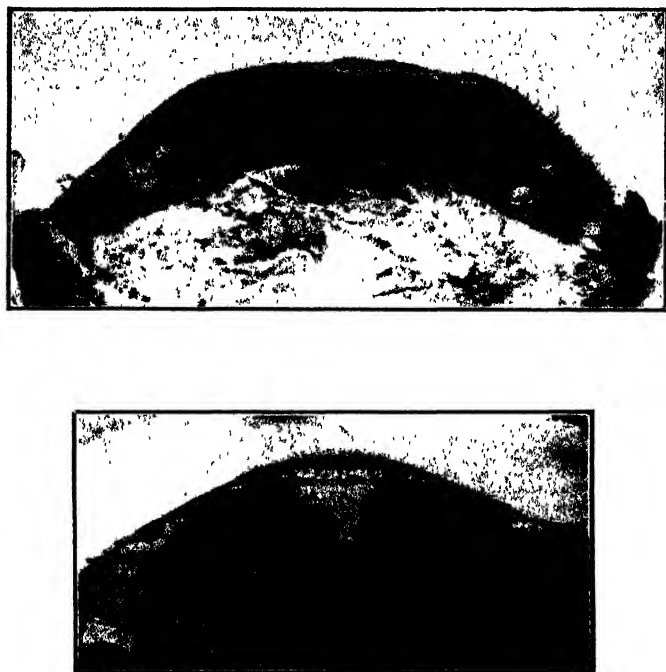
FIG. 4.
Necrotic lesions on lower lips.

(a) *Above:* In a natural cattle case, note impressions of incisor teeth.
(b) *Below:* In an experimental sheep case (Sheep 8, Expt. 1b). Note lesions opposite lateral incissors.

The tongue is swollen and red. In some cases it is even bluish (cyanotic) in colour. It may be enlarged to such an extent that it protrudes from the mouth and 7 or 8 inches of the organ may be exposed. This protruded portion becomes dry. The tongue appears to be partially paralyzed in such instances since it cannot be properly held in its normal position. The lingual papillae are dark red in colour and on the ventral surface of the apical portion extensive necrotic lesions are usually to be found (*vide* Figs. 5 and 6).

In some cases the mucosa along the frenum linguae only is affected and such lesions are usually linear in shape.

Deep seated and extensive necrotic lesions may be present on the tongues of some individuals. These lesions are usually found on the dorsal and lateral aspects of the middle portion.

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FIG. 5.

Onlangs Case· Note extensive necrotic lesion on ventral aspect of tongue.

FIG. 6.

Novo Case: Necrotic lesion on ventral surface of tongue in stage of healing.

When the necrosis on the dental pad is extensive, it is usually confluent with similar lesions on the upper lip. The injured mucosa has a distinct grooved appearance at the junction of the lip and pad.

Lesions are sometimes evident on the gums and these are usually present on the floor of the mouth just posterior to the incisor teeth.

Where such extensive mouth changes are present the odour of the breath is very offensive.

Healing commences after about 3 or 4 days. The muzzle now becomes covered with a thick scab, which subsequently peels off leaving a clean and fresh looking surface without formation of cicatrices. The lesion on the ventral surface of the tongue takes somewhat longer to heal. In the later stages a peculiar wrinkled appearance is noticeable (*vide* Fig. 7).

FIG. 7.

Montague Case: Necrotic lesion along the frenum linguae in advanced stage of healing.

The deep seated necrotic ulcers on the tongue take a longer time to heal and all the processes of repair can be followed.

The teat and udder symptoms.—Lesions of the teats are only observed in cows which were actually in milk.

The teats become markedly inflamed and where pigmentation is light or absent, they are dark red in colour. The surfaces are raw and any handling is intensely resented. After a few days a hard and fairly thick scab is formed and this in time peels off *in toto* like the finger of a glove. The newly formed epithelium then has a fresh and clean appearance.

Small localized skin lesions are sometimes found on the udder. These lesions are usually about 1 cm. in diameter and are covered with a soft yellow scab, which, when removed leaves a moist surface and a slight reddening of the underlying tissues. The entire skin of the udder is sometimes involved in an extensive dermatitis. These extensive skin changes will be described under the next heading.

FIG. 8.

Montague Case: Sloughing of epidermis underneath the tail, etc.

The skin changes.—Skin lesions are usually observed in severe reactions. In mild affections they may be entirely absent or localized to the thinner portions, e.g. in the flanks, at the root of the tail, on the udder, etc. (*vide* Fig. 8).

During the early stages symptoms of an acute dermatitis are present. The skin is reddened on the unpigmented portions. It appears swollen and is painful, especially along the back. A small amount of straw coloured exudative material collects on the surface. This exudate dries and the basal portions of the hairs become matted together. Soon hard crusts and scabs are formed. The hardened condition in this stage resembles the condition of the skin observed in the later stages of sweating sickness of calves ,*vide* du Toit, 1923.)

A distinct photosensitivity becomes evident and such affected animals are usually found sheltering underneath trees or in the shade of a wall or a shed. When driven out of such places they immediately seek another shady locality. The scabs are at first firmly adherent, and if forcibly removed the underlying tissues are found to be moist and bleed easily. Healing progresses and later these scabs peel off. The hair comes away with the desquamated epidermis.

In the severe reactions the dermatitis is fairly generalized (*vide* Figs. 9, 10, 11).

FIG. 9.

Kromdraai Case: Extensive dermatitis followed by hardening of the skin and the formation of scabs.

FIG. 10.
Kromdraai Case: Extensive skin lesions on udder, etc.

FIG. 11.

Kromdraai Case: Note the formation of crusts and scabs on skin of muzzle.

It will be noticed that a lesion in the case at Montague (*vide* Fig. 12) is confined to an unpigmented portion of skin of the upper portion of the flank.

FIG. 12.

Novo Case: Skin lesions confined to an unpigmented portion of the skin.

The feet lesions.—It has been mentioned that lameness and stiffness are usually the first symptoms which become evident. On examination the lower portions of the limbs are found to be swollen, the swelling usually extends from the coronet to the fetlock and all four feet are affected to the same extent. In some cases the swelling may extend to the knees and hocks. The skin, particularly on the plantar surface in the region of the accessory digits is reddened and a small amount of straw-coloured exudate is usually present on the surface.

In a number of cases an excoriation of the epidermis in the interdigital space was observed. In such instances partially detached epidermis may still be found to be adherent to the excoriated areas and this may convey the impression of a recently ruptured vesicle.

Usually the hyperaemia and swelling of the skin disappears without further changes, but frequently the dermatitis progresses and later lesions develop on the skin of the digit similar to that described in other regions.

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Rarely (*vide* Fig. 13) a separation of the claws along the coronary bands becomes evident. In such cases the anterior portion of the interdigital space present a raw and granulating surface. It

FIG. 13.

Verblyden Case: Note fissures at the coronets.

would appear that this type of lesion is apt to develop in cases which are forcibly walked during the acute stages. This lesion would appear to be due to a coronitis, as is observed in sheep.

According to a report received from a farmer (*vide* Appendix C), exungulation may occur.

Eye lesions.—Usually no eye lesions are noted, but in some instances a slight watery discharge may be present which later becomes catarrhal.

(2) *Atypical Cases.*

(a) *Cases resembling three-day-stiffsickness.* — Keppel and Robinson (*loc. cit*) state that the symptoms noted in their " ulcerative stomatitis " to some extent resembled three-day-stiffsickness. Further in an outbreak at Oudehoutsdraai, Volksrust (*vide* Appendix C) Williams and Dickson encountered two typical cases of this disease. In this particular herd several more animals were observed which, according to them, were showing symptoms of three-day-stiffsickness. They did not find any mouth lesions in these cases.

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It would seem very probable that such cases of so-called " stiffsickness " could be due to the same cause, i.e. bluetongue virus. The only symptom of a reaction which became evident, was a rise in temperature and stiffness. These cases may be compared with the " abortive " reactions commonly noted in sheep, where the only indication of a reaction is an elevation in body temperature.

(*b*) *Peracute reactions.*—Only one instance of a fatal peracute reaction in cattle came to our notice. The symptoms observed in this case are fully described in Appendix A (experiment 4).

This animal died within three days after the first symptoms became obvious.

The following changes are of interest and importance with regard to the symptoms and lesions which may be noticed in such a peracute reaction :—
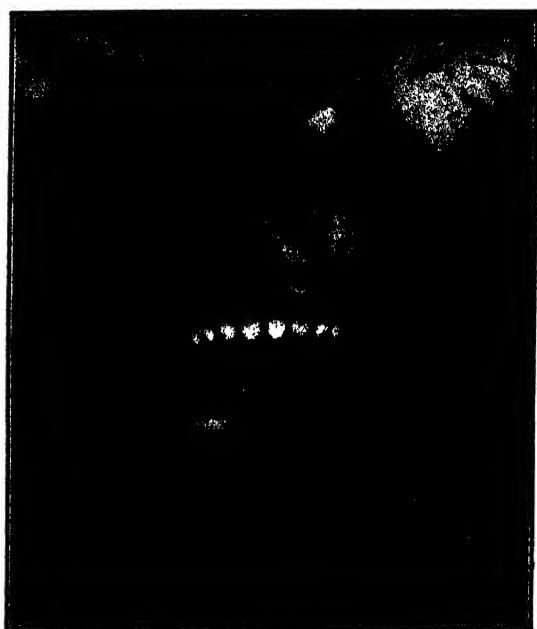
. FIG 14.

Elandslaagte Case: Necrotic lesions on gingiva (peracute reaction).

(1) The marked hyperaemia, and the presence of numerous petechiae and ecchymoses in all the visible mucosae, e.g. of the nostrils, lips, hard palate, tongue, cheeks and conjunctiva.

(2) The marked muco-haemorrhagic nasal discharge and slight epistaxis.

(3) Superficial localized inflammation with necrosis on the buccal mucosa, e.g. on the gingiva (*vide* Fig. 14), dental pad, hard palate, etc.

(4) The swelling of the vagina (*vide* Fig. 15), and the marked hyperaemia of the vaginal mucosa with numerous petechiae and ecchymoses.
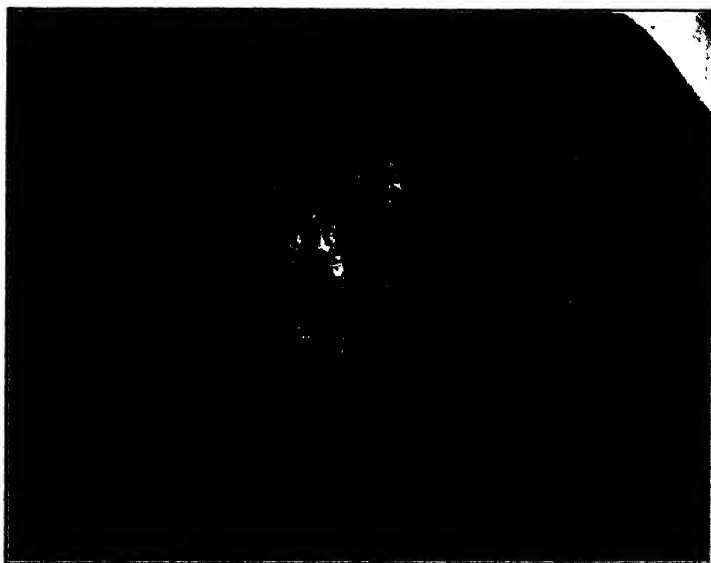
FIG. 15.

Elandslaagte Case: Swelling of vagina and skin lesions (peracute reaction).

(5) The swelling of the lower portion of the limbs.

(6) The presence of superficial skin lesions (*vide* Fig. 16).

FIG. 16.

Elandslaagte Case: Skin lesions on metatarsal region (peracute reaction).

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(b) As Observed in the Experimentally Infected Calves.

A considerable number of calves were used in the transmission experiments (*vide* Appendix A). In some of these no perceptible reactions could be ascertained, but in the majority definite and constant changes were observed. These reactions were mild in comparison with the very severe symptoms and lesions seen in natural infections, but were sufficiently evident to enable one to conclude that the calves reacted to the virus.

The first symptom usually noted is a rise in body temperature which is, of course, indicative of a general or systemic reaction. A number of representative temperature charts are included (Appendix D) and may be referred to (*vide* Charts VIII to XI). There is an incubation period of from two to three days.

With the rise in temperature lesions appear with constant regularity on the buccal mucosa. They were found to be usually confined to the mucosa of the upper and lower lips. These lesions commence as small raised areas, about the size of a millet seed. Their centres have usually a yellow colour and the surrounding tissues are slightly injected. Soon the epithelium appears to become destroyed and excoriations result. These excoriated areas can become quite extensive (*vide* Fig. 17).
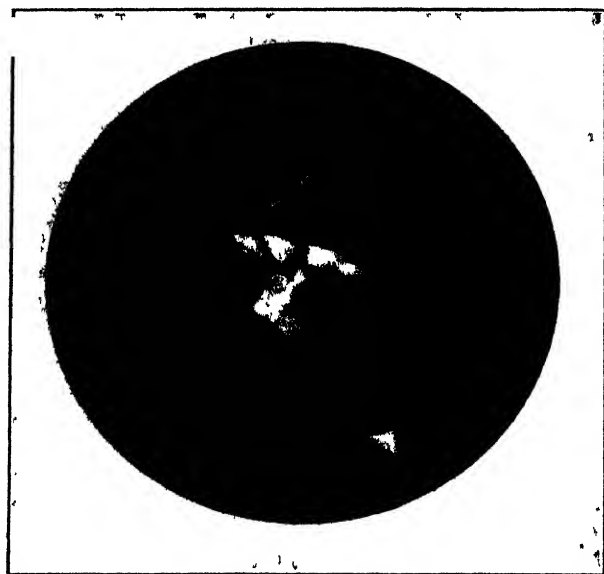
FIG. 17.

Calf No. 5407: Lesions on the lips observed in transmission experiments in calfs.

Frequently the loosened epithelium is still partially attached to the borders of the lesions, which resemble recently ruptured vesicles. It should be noted, however, that the formation of actual vesicles was never observed. The lesions have an irregular shape

and may be longitudinal, circular, oval, etc. They occur with marked regularity opposite the lateral incisors on the inner surface of the lower lips, but are also frequently seen on the borders of the lips and at the commissures of the mouths.

In a few instances such lesions developed without any noticeable rise in body temperature.

The nasal mucosa was sometimes also involved and the excoriations were usually present on the septum nasi. In these cases a slight catarrhal nasal discharge was evident. The lesions usually persist for about four or five days. Healing then commences and after about 10 days they disappear completely.

In several instances hyperaemic areas were seen on the hard palate and in a few a distinct diffuse injection of the mucosa of the lips, cheeks, etc., become noticeable. This was particularly evident in calf 5263 [vide experiment 1 (b)]. The hyperaemia of the buccal mucosa was first noticed six days after the day of inoculation. On the next day the lower lip appeared swollen and reddened with a few petechiae. In addtion the apical portion of the tongue was reddened and a few of the papillae distinctly enlarged and dark red. The ventral surface of this part of the tongue was excoriated in several small areas where the underlying tissues had a slightly granulated appearance. Excoriations and numerous petechiae were also noted on the borders of the lips. Several of the conical papillae of the lips and cheeks appeared enlarged and reddened while the tips were yellowish grey. The picture of the mouth as seen in this calf resembled that of typical cases in natural outbreaks of a mild nature.

Several of the experimental calves became visibly ill. They stopped feeding and lay down frequently. Fairly extensive ulcers developed on the tongues of a few of these experimentally infected calves (vide Figures 28 and 29).

In one calf [No. 10 of experiment 1 (b)] a marked dyspnoea developed on the 12th day after infection. The animal was destroyed and very extensive necrosis and ulcers were found on the lateral aspect of the tongue and in the larynx. Similar lesions were noted in some of the experimentally infected sheep.

No feet or skin lesions were observed in any of these calves.

(c) As Observed in Experimentally Infected Sheep.

The sheep used in the transmission experiments were kept under very close observation and provided suitable material for studying the course and symptoms of the disease in sheep. Spreull (loc. cit.) and Theiler (1905) have described the symptoms of bluetongue in sheep in fair detail and our observations on the reactions produced by virus recovered from cattle do not materially differ from theirs.

The incubation period after artificial infection.—This was found to vary considerably as will be evident from an analysis of the incubation periods of the following 66 cases:—

No. of days after inoculation.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
A.—Sheep infected with virus recovered from cattle. No. of cases.....	1	7	9	6	9	8	5	1	1	1
B. Sheep infected with virus recovered from sheep. No. of cases...	—	1	1	3	7	4	1	1	—	—

It will be noticed that the incubation period may be as short as one day and may be delayed for as long as 10 days. The onset of a reaction is usually indicated by a rise in temperature, but occasionally an injection of the mucosa of the lips and slight frothing at the mouth become noticeable before the temperature rises.

The Temperature.—Generally the temperature rises gradually and the acme is reached after three or four days, but sometimes a very sudden pronounced rise takes place, and in such instances the acme may be reached within 24 hours. The fever is usually of a *continued* type, lasting for 6 or 7 days, but temperature reactions of a distinct *intermittent type* are quite commonly noted. In such cases the period of apyrexia does not usually last longer than from 24 to 36 hours. In a small percentage of cases the temperature reaction is of very short duration, in fact some of them may be regarded as *ephemeral.*

In a few cases severe clinical reactions were observed without the occurrence of a rise in body temperature. Such reactions may be considered as *afebrile.*

Typical temperature charts are included (*vide* Appendix D, Charts I to VII).

The Habitus.—In the early stages only slight disturbances can be observed. The sheep usually stop feeding and ruminating. They lie down frequently and peculiar chewing and licking movements become noticeable. After the rise in temperature dyspnoea becomes fairly evident and the nostrils are dilated. As the reaction progresses dullness becomes evident; the head hangs down and the ears droop. When the temperature is very high the animals drink water frequently. In severe reactions the sheep lie down continuously and assume the attitude noted in Fig. 18.

The lesions and symptoms in the mouth and nose.—An injection of the mucosa is usually seen soon after the commencement of the temperature. The hyperaemia is particularly noticeable on the inside of the lips which assume a cherry-red colour. The redness may extend to the skin and the lips and nose becomes distinctly pink. The lips now become swollen and in some cases even oedematous (*vide* Fig. 19).

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FIG. 18.

Sheep 37812. Characteristic attitude assumed during severe acute stage.

FIG. 19.

Sheep 37812: Marked swelling (oedema) of lower portion of the head.

The nostrils and muzzle are at first dry, but a catarrhal discharge soon develops. This usually changes to muco-haemorrhagic. When the discharge dries, thick incrustations are formed at the nasal orifices, and these interfere considerably with breathing.

The mouth is now extremely painful and handling is resented.

Petechiae and ecchymoses now become noticeable on the buccal mucosa and are particularly evident on the lips, tongue, nose, palate, dental pad and cheeks.

The petechial stage is soon followed by the appearance of excoriations of the mucosa. These lesions are usually localized and vary in extent. They occur regularly on the following sites: borders of the lips; on the inner surfaces of the lips opposite the lateral incisors (c.f. in experimentally infected calves); at the commissures of the mouth; on the ventral surface of the tongue (particularly along the frenum linguae); the dental pad; on the gums (especially on the portion posterior to the incisor teeth); on the lateral surfaces of the medial portion of the tongue and inside the cheeks opposite the molar teeth. The conical papillae of the lips also become red and the tips assume a greyish-yellow colour. At this stage a cyanosis usually becomes evident.

FIG. 20.

Sheep 37328: Lesions on lips, muzzle and nose. Note slight catarrhal discharge eyes.

The *typical blue condition of the tongue* from which the name of the disease is derived, is seen when the following changes are present in this organ: swelling, hyperaemia and cyanosis, petechiae and ecchymoses and the initial stages of an inflammation of the mucous membrane. Sometimes the tongue becomes markedly swollen and in such cases an oedema of the subcutis is usually also evident. The skin of the nostrils, lips and muzzle also becomes excoriated and scabs are formed (*vide* Fig. 20); when the encrusted discharge from the nostrils or the scabs covering the excoriated lesions are forcibly removed the underlying tissues present a granulated appearance.

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When the mouth is manipulated, bleeding from the injured buccal mucosa can be easily provoked. When these lesions are present, the breath is foetid. When recovery sets in, the injured mucosa heals fairly rapidly, but fairly deep-seated ulcers may develop on the tongue and these take a considerable time to heal (*vide* Figs. 27 and 29).

Skin Lesions.—The skin of the lips and muzzle is usually injected, but frequently a redness of the ears and at the bases of the horns becomes noticeable and in a few instances the entire skin is somewhat flushed.

Changes in the Eyes.—The conjunctiva sometimes becomes slightly affected and petechiae and ecchymoses are occasionally noted. Slight lachrymation is seen in such cases and in the later stages a small amount of catarrhal discharge may be present.
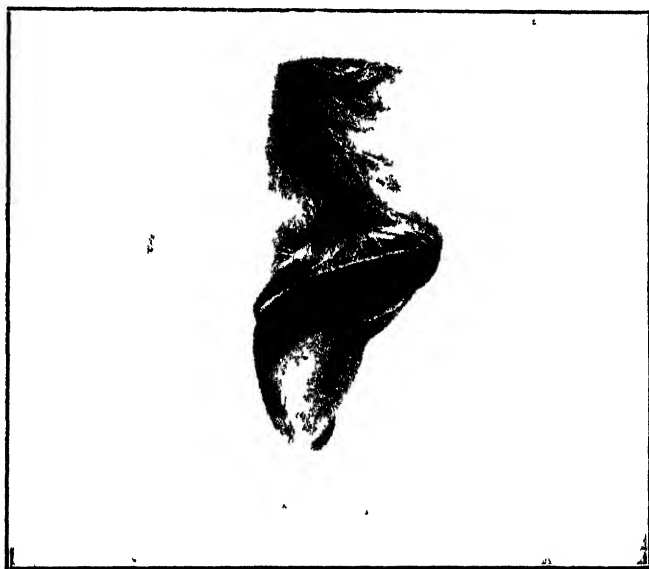
Fig. 21.

Sheep 37071: Partial exungulation of claws (three months after infection).

The vagina usually becomes swollen and reddened with petechiae and ecchymoses. Frequently the borders assume a dark bluish-red colour and later excoriations may even develop.

The Feet Lesions.—On about the 9th to the 12th day after the onset of the reaction a marked coronitis develops. The claws first become hot and soon the coronary bands assume a dark red colour. This redness usually extends to the bulbs. The coronitis may persist for about a week, but frequently it disappears after a few days. The feet lesions usually develop after the temperature has subsided and when the mouth lesions have begun to heal. The animal lies down frequently and when forced to walk the gait is distinctly laminitic. No instances of complete exungulation were noticed, but in several

instances deep grooves (partial exungulation) became noticeable (*vide* Fig. 21) on the claws. These feet lesions were noted in about 50 per cent. of the cases.

Henning (*loc. cit.*) states that one, two or three, or all feet may become affected and, according to this author, a correlation is said to exist between the severity of the feet and mouth lesions, he makes the following statement: " Sometimes the feet of animals suffering from a comparatively mild attack of bluetongue become seriously affected, whereas the feet are seldom badly inflamed in those cases where extensive mouth lesions have developed ". This phenomenon was not observed in these experimental cases and furthermore when a coronitis developed all four feet were found to be affected to the same extent.

Apart from the cessation of feeding and rumination no other serious digestive disturbances became manifest in the majority of cases. In a few instances, however, diarrhoea developed shortly before death.
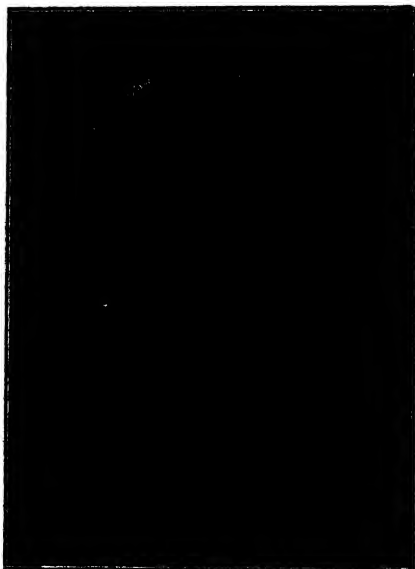
Fig. 22.

Sheep 37085: Torticollis, a symptom frequently observed in severe reactions.

There is a rapid loss of condition and it takes a considerable time for the sheep to recover the loss of flesh. In a number of cases marked debility followed the reaction and some of the animals became so weak that they had to be destroyed. The mouth and feet lesions had by this time completely healed. [In several cases a marked *torticollis* developed prior to death (*vide* Fig. 22.]

Frequently a swelling (oedema) of the head and neck developed and the condition was not unlike that noticed in dikkop horsesickness. In these cases a further complicating symptom was the discharge of ingesta through the nostrils (*vide* Fig. 23). Presumably this is due to a paresis of the oesophasus. Theiler (1918) describes such a complication in horsesickness

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The following stages in the course of the disease can be distinguished :—

Firstly, an elevation of body temperature, which is followed in a day or two by a swelling of the lips and redness of the buccal mucosa. Petechiae and ecchymoses now appear, to be followed soon by a localized inflammation, necrosis, and excoriation of the mucosa.

During the stage of severe mouth lesions the temperature commences to drop. After about the 10th day following the onset of the reaction the coronitis appears.

FIG. 23.

Sheep 37328 : Note lesions on lips and muzzle, also a marked discharge of mucus and ingesta from nostrils and oedema of head.

The above course may at any stage be interrupted by either recovery or death. Thus in some sheep a hyperaemia of the buccal mucosa may be the only clinical condition that becomes perceptible, whilst in others localized in flammatory lesions with necrosis also become evident, but no feet lesions develop. The experimentally infected sheep died at irregular intervals after the date of infection as is indicated in the following table :—

No. of days after inoculation	5	6	7	8	9	10	11	12	13	14	16	17	18	19	20	21
No. of sheep which died on this day	—	2	3	—	2	1	1	3	5	3	1	1	3	—	1	—

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It is possible to distinguish : —

   (a) *Peracute reactions*, where the reaction ceases (either by death or recovery) with hyperaemia or petechiae;

   (b) *acute reactions*, where the reaction resolves itself into a localized inflammation with necrosis of the buccal mucous membrane, the animal either dying or recovering in this stage;

   (c) *subacute*, where a coronitis develops, or where the animals become very debilitated and may even die.

     In these later stages a paresis of the oesophagus and the formation of extensive oedema frequently becomes evident.

Partial or complete shedding of the wool was observed to occur in a few of the experimental animals. This may be considered a sequel of the reactions and such a shedding of the wool is also seen in field outbreaks of bluetongue.

(d) A COMPARISON OF THE SYMPTOMS IN CATTLE AND SHEEP.

With reference to:	In *cattle* as seen in natural outbreaks.	In experimentally infected calves.	In experimentally infected sheep.
The incubation period	Not known	About 4 days	Varies considerably, but usually about 4–5 days. May be as short as 24 hours and as long as 10 days.
The temperature....	Temperature of more than 106° F found in several cases. Most cases, however, came under observation in the later stages, when the mouth lesions are well developed and temperature had apparently already subsided	Definite temperature reactions observed in many cases. A maximum temperature of 106° F recorded in several instances. In some of the calves no serious disturbance in body temperature noted	Temperature reaction a very constant and usual feature of the reactions. Occasionally an afebrile reaction encountered.
Stiffness, lameness, and swelling of lower parts of the limbs in the early stages	A feature observed in the majority of cases. Sometimes the first symptom noted by the owners	Not observed	Not observed.

With reference to:	In *cattle* as seen in natural outbreaks.	In experimentally infected calves.	In experimentally infected sheep.
The oral changes	Consist essentially of a hyperaemia of the mucosa and localized inflammation with necrosis of the mucous membrane. Particularly evident on the following sites : upper and lower lips ; gums and dental pad ; tongue (especially on the ventral surface of apex); muzzle and external nares. In one case (considered as a peracute reaction) petechiae and ecchymoses predominated	Hyperaemia was generally localized, but in a few cases diffuse hyperaemia of the buccal mucosa noted. Usually localized excoriations develop on the inner surface of the lips. On the lower lip these lesions appeared with marked regularity opposite to the lateral incisors. In one case a re-production of the changes in natural outbreaks in cattle was reproduced but of a mild nature	These changes commence with a diffuse hyperaemia of the buccal mucosa, especially of the lips. The hyperaemic stage is soon followed by the appearance of petechiae and ecchymoses and soon excoriations and necrosis of the mucous membrane develop. These lesions are particularly evident on the following sites : lips, tongue (especially along the frenum linguae), inside of cheeks, dental pad, gums, muzzle, and external nares.
Deep seated necrotic ulcers on the tongue	Such necrotic ulcers were very marked in two of the cases encountered on the farm Welgezegend	Observed in at least two experimental cases	Observed in several sheep and here it can be definitely stated that these ulcers developed from the usual superficial necrotic process.
Discharge from nostrils and subsequent incrustation	The discharge usually mucoid or mucopurulent, and marked encrustations formed. A muco-haemorrhagic discharge observed in one case	A watery discharge, sometimes slightly mucoid noted in several cases	The discharge is first mucoid but soon becomes muco-haemorrhagic. In a few cases ingesta passed through the nostrils. Marked formation of incrustations.
Salivation..........	Very marked in cases where tongue protrudes. Marked frothing and slight salivation is a usual feature	Slight frothing observed in one or two cases	Frothing at the mouth common symptom, especially in early stages.
Discharge from the eyes	A slight catarrhal discharge noted in a few cases	A watery discharge (lachrymation) seen in a few	Watery discharge observed in some cases with a tendency to become catarrhal in later stages.

With reference to:	In *cattle* as seen in natural outbreaks.	In experimentally infected calves.	In experimentally infected sheep.
Teat lesions	Regularly observed in lactating cows. The teat lesions consist of marked reddening of the skin, destruction of the epidermis and later the formation of hard scabs	Not observed, but suitable animals not available	Not observed. Note: Only two ewes in milk inoculated, but unfortunately both developed per-acute fatal re-actions.
Localized skin lesions on the udder	Noted in a few lactating cows. Lesions about 1 cm. in diameter, covered with yellow scab and the underlying tissues slightly red or moist	Not observed......	Not observed.
General skin lesions	Very marked skin lesions, either localized to thinner portions of the skin, or diffuse and general, a common, although not constant symptom. These skin lesions develop in the later stages and consist of reddening, slight exudation, later a hardening and formation of crusts, which in course of time sloughs off, the hair also coming away	Not observed......	A reddening of the skin of lips and nose frequently observed. Some times also of the ears and in rare cases the entire skin becomes flushed. Shedding of the wool as a sequel.
The vulva.........	Swelling of the vulva with petechiae and ecchymoses observed in one case	Not observed......	Swelling of the vulva with necrotic changes on the borders and petechiae and ecchymoses in the mucosa commonly observed.
Oedema of subcutis	According to Robinson and Keppel (*loc. cit.*) a swelling of the neck was observed in one of their cases. A case at Elandslaagte showed a distinct swelling of the lower portion of the head	Not observed......	Very extensive sub-cutaneous oedema of the head and neck observed in several animals. In these cases a " paralysis " of the oesophagus frequently ensued.

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With reference to :	In *cattle* as seen in natural outbreaks.	In experimentally infected calves.	In experimentally infected sheep.
Swelling of the tongue	Very marked in several cases which results in a protrusion of the tongue from the mouth	Not observed......	Extensive swelling of the tongue seen in several cases, but actual protrusion of the tongue not very evident.
Torticollis.........	Not observed......	Not observed......	Fairly frequent symptom generally noticed some hours before death.
Intestinal disturbances	Not observed......	Not observed......	Diarrhoea observed in a few instances.
Claws.............	In one case a distinct separation of the skin and claw of the coronets was observed. In this case all four feet were affected. Whether this was preceded by a hyperaemia of the coronets (a very characteristic symptom in sheep in the later stages) is difficult to say. In several cases an excoriation of the epidermis in the interdigital space was seen. (Note : exungulation has been reported by a farmer, *vide* Appendix C)	No changes observed	A hyperaemia and an acute inflammation of the coronets developes 12–14 days after infection. The coronets become dark red in colour and the redness extends to the bulbs. The animals become very lame. Partial exungulation was observed.
Debility in the later stages	Noted in a few cases. Such animals were found in a moribund condition and destroyed	Not seen..........	Noted in several cases.

PATHOLOGY.

In Appendix B a summary will be found of the most important macroscopical and microscopical changes observed in the *natural cattle cases* killed for post-mortem examination and in the *experimental sheep cases* which died or were killed during different stages of the disease. An attempt will be made to classify the characteristic pathological changes in the different organs, and to compare these with the observations of former investigators (Theiler and Spreull).

Localized hyperaemia of an active type or *diffuse hyperaemia* of a venous nature was seen in the skin, especially on the teats of the udder, the tongue, lips, rumen (including the oesophageal

groove), reticulum, omasum, abomasum, large and small intestines, larynx, trachea, pharynx, myocardium, kidneys, liver, nasal mucous membrane. It was significant how seldom the spleen was affected in the experimental cases. Where tumour splenis occurred it was usually associated with a complication (e.g. a sequel of perforation of the rectum). It was only in the later stages of the disease that hyperaemia and inflammation of the coronets revealed themselves.

Multiple haemorrhages varying from petechiae to suggillations occurred frequently in connection with the skin, the lips, the mucous membrane of the lips, tongue, dental pad, buccal cavity, small intestine, myocardium, epicardium and endocardium, less frequently in the trachea, nasal cavity, bladder, urethra, pulmonary artery, pleura.
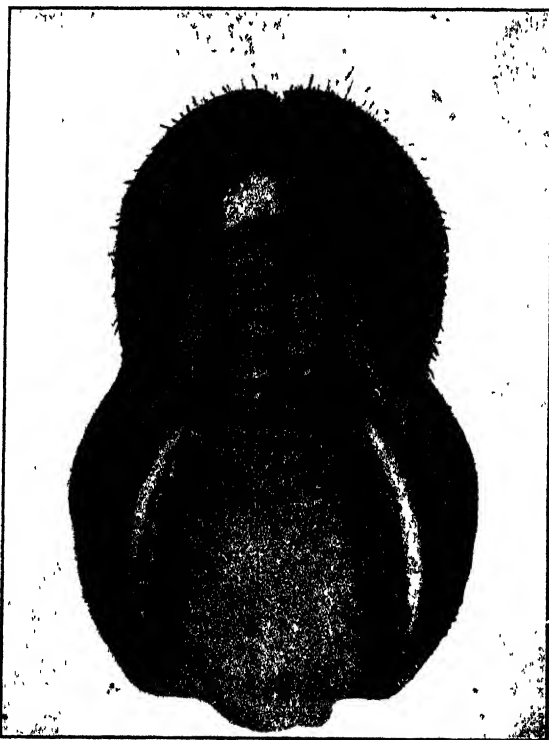
FIG. 24.
Swelling of lip and multiple haemorrhages with necrotic lesions along borders of lip, inside of cheeks, hard palate, etc. (From drawing.) Sheep 37091.

These *haemorrhages* were usually multiple, circumscribed and about $\frac{1}{2}$ cm. in diameter. They were confined to the mucous membranes, but in the case of the heart they were also seen in the substance of the myocardium. In case of the epicardium in a large number of cases the apex showed a diffuse reddish patch about 1-1$\frac{1}{2}$ cm. in diameter, more or less encircling the apex. Interesting were also the circumscribed haemorrhages around the root of the hairs within the follicles of the skin.

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The localized necrotic areas followed by ulceration were usually seen on the lips opposite the incisor teeth, the dental pad, at the apex of the tongue, on the ventral aspect, and usually on the dorso-lateral aspect of the tongue opposite the fourth molar teeth, the mucous membrane of the rumen, the pylorus of the stomach, the external nares. (*Vide* Figs. 24–30.)
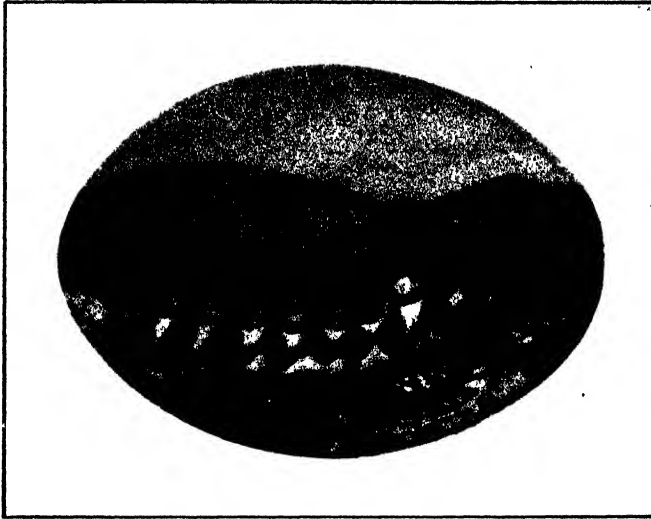
FIG. 25.
Necrotic ulceration on lateral aspect of tongue and cheeks, particularly marked opposite prominent molar teeth. Sheep 37691.
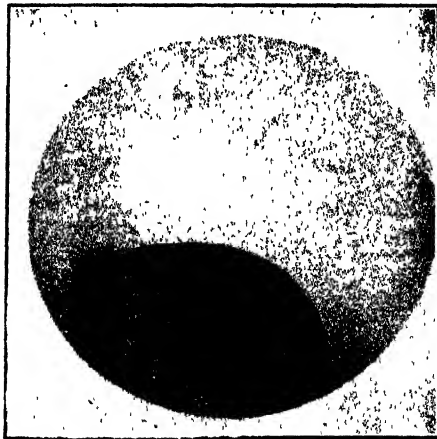
FIG. 26.
Necrotic ulceration along frenum linguae. (Sheep 8, Expt. 1B.)

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1   2   3.

FIG. 27

Various stages of ulceration on tongues of sheep
1 Sheep 8 of Expt 1 (b)   13 days after infection
2 Sheep 1 of Expt 1 (a)   9 days after infection
3 Sheep 12 of Expt 1 (c)   13 days after infection

FIG. 28.

Extensive ulcer lateral aspect tongue   Calf 10, Expt 1 (b)
18 days after infection

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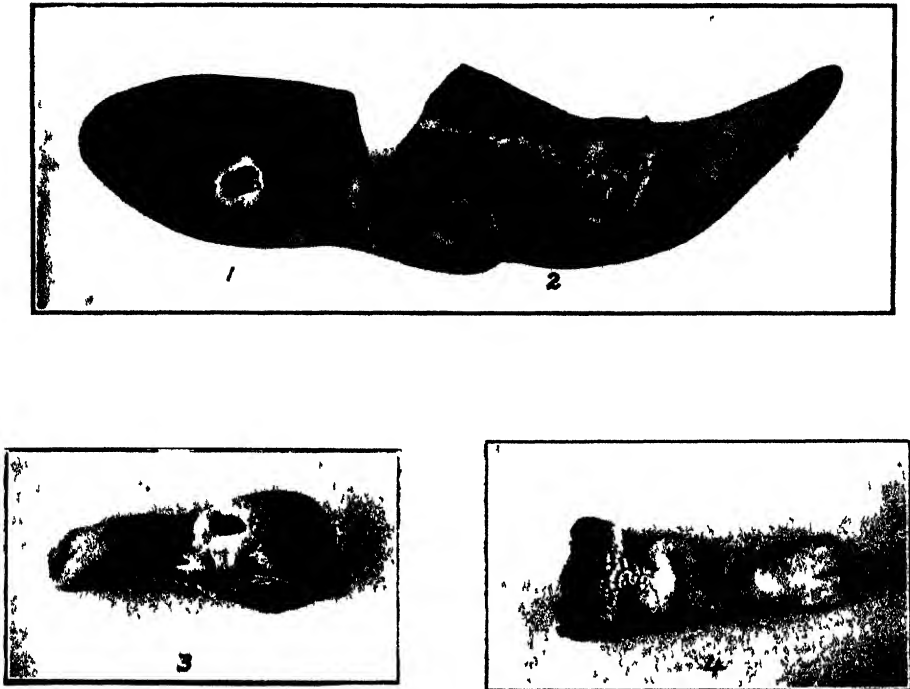
FIG. 29.

Ulcers on tongues in the process of healing.

1 Calf 7, Expt. 1 (b), 20 days after infection.
2. Calf 1, Expt. 1 (a), 27 days after infection.
3 Sheep 17, Expt 2, 25 days after infection.
4 Sheep 16, Expt. 2, 25 days after infection.

Microscopically these lesions in the acute stage could, in the majority of cases, be distinguished from the vesicles of foot and mouth. They either showed a necrosis or a subsequent ulceration with an hyperamic zone. At the periphery of the ulcer one could always recognize the presence of some of the adhering necrotic material. At no stage of the disease did it disclose anything of the nature of the frayed out and irregular remains of the wall of a ruptured vesicle of foot and mouth. Neither was the base of the ulcer like the usual foot and mouth lesion, namely, of a bright red granulating surface. These necrotic areas or ulcers were circumscribed, well defined, and often multiple. In the natural cases of bovines at Welgezegend some of the necrotic areas on the tongue reached large dimensions, and penetrated into the muscular substance of the tongue. In one case it revealed multiple necrotic foci in the lung, but apparently caused by *B. necrophorus*.

3   433
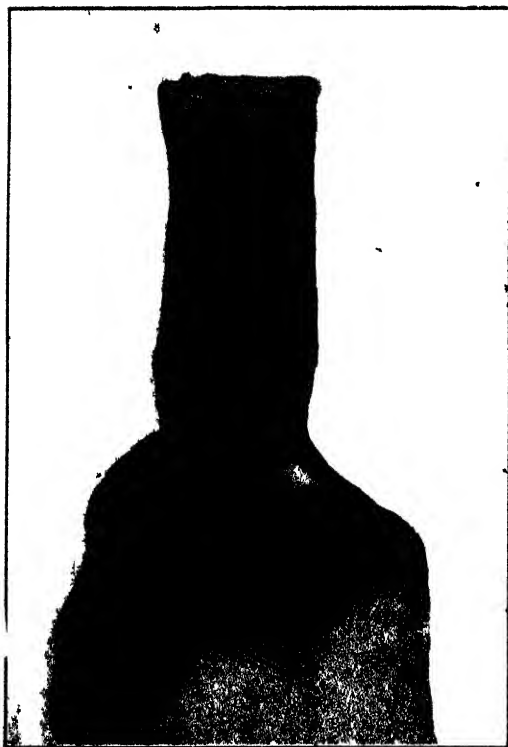
FIG. 30

Acute enteritis and haemorrhages in pyloris (from drawing)   Sheep 37454.
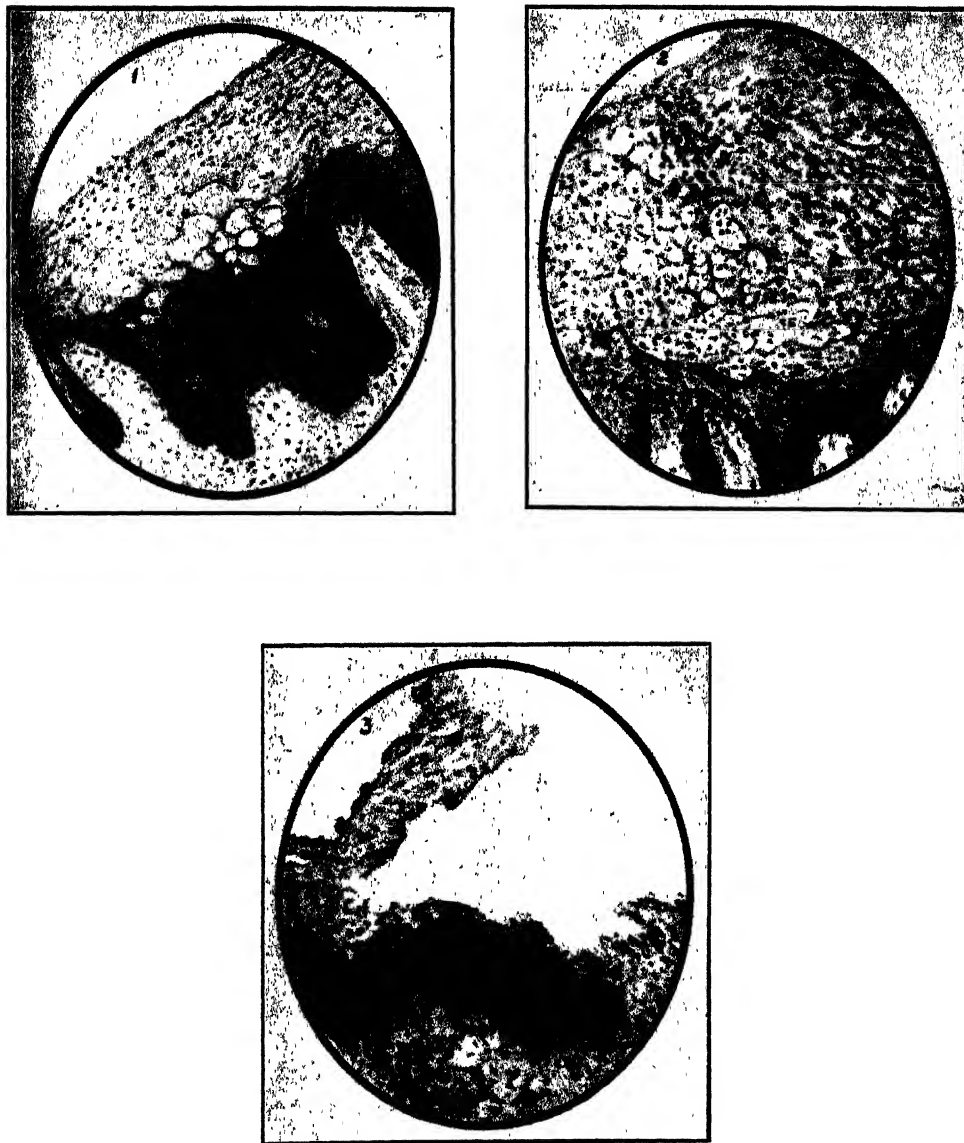
Fɪɢ. 31.

Tongue, showing three stages of bluetongue lesions.
(Spec. 13961, sheep expt. case.)

1. " Ballooning " of stratified epithelial cells.
2. Infiltration of epithelial cells with neutrophiles.
3. Desquamation of necrotic material forming ulcers.

FIG. 32 (1).
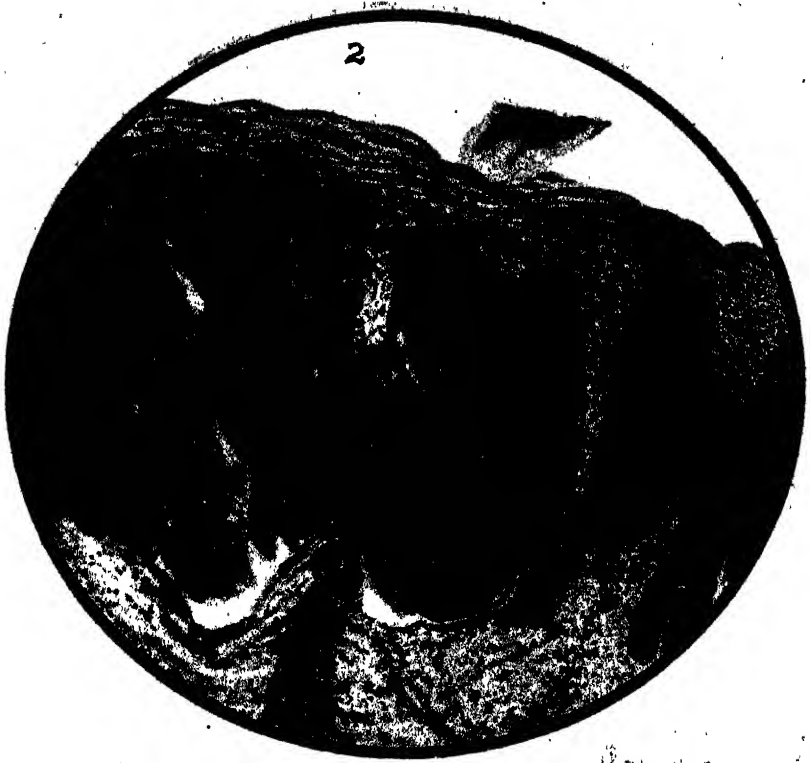
FIG. 32 (2).

Tongue: Haemorrhages in stratified epithelium and corium of mucous membrane. (Spec. 14065, Expt. sheep case.)

Microscopically (*vide* Figs. 31 and 32), it was seen that these necrotic areas and ulcers were produced by a process of " ballooning " of the epithelial cells of the stratified epithelium of the mucous membrane of the *tongue, lips, rumen, etc.* This was followed by a pustular stage in which the spaces in the epithelial cells became loosely infiltrated with neutrophiles. This was followed by necrosis and ulceration of the mucous membrane in which the corium becomes secondarily involved. The underlying corium revealed hyperaemia, haemorrhage, infiltration with neutrophiles, and necrosis. In all these cases the base of the ulcer was formed by a necrotic mass, practically unassociated with any proliferation of connective tissue.

The healing process sets in rapidly; but frequently, however, well defined depressions develop in the mucous membranes, about $1\frac{1}{2}$ cm in diameter to $\frac{1}{8}$ in depth with the edges sharply cut and clean. At this stage the lesions may be mistaken for healing-out lesions of foot and mouth and like in case of foot and mouth it is surprising how rapidly and completely healing of the mucous membranes takes place.

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Oedema of the lips and tongue was an early lesion to occur and was usually fairly prominent, in some cases the tongue protruded from the mouth as a result of its increased size, due to swelling. The subcutis of the ears, supra-orbital fossae, mandibles, inter-mandibular space, ventral aspect of the upper third of the cervical region, the peritracheal and perioesophageal connective tissues were also frequently affected.

In a number of cases there was oedema of the glottis and lungs, as well as a hydropericardium, and hydrothorax. In sheep 37452 oedema of the glottis was so marked that it caused asphyxia. Some of these cases in view of the transudation of fluid into the connective tissues of the cranial aspect of the body resembled the dikkop form of horsesickness.

Some cases showed inflammation of the intestines, urinary bladder, and degenerative changes in the parenchymatous organs. A large number of these were examined microscopically, e.g. brain, spinal cord, liver, kidneys, spleen, a large number of lymphatic glands without evidence of any specific changes.

Some animals that died in the later stages of the disease showed general anaemia and cachexia as the dominant features.

In describing the lesions found in bluetongue in sheep Theiler (*loc. cit.*) maintains that one can consider as typical only those found in such cases where death supervened in the acute stage. He says, " The mouth is the principal part affected, showing excoria-tions on the lips, gums of the upper jaw, sloughing off of the epithelium of the tongue . . . . mucous membrane of the nasal septum is usually strongly congested . . . . lesions in the first stomach showing red patches and stripes . . . in severe cases the mucous membrane of the fourth stomach is of a purple colour, swollen, either uniformly discoloured or perhaps in patches. . . . Lungs are as a rule normal, but symptoms of even complete oedema are occassionally met with. . . . In acute cases tumefaction of the spleen is usually present, but does not reach large dimensions. . . . Petechiae are found in almost every acute case on the left endocard and sometimes on the epicard.

The post-mortem lesions in cases of long duration leave nothing typical. Usually there are lesions of extreme emaciation, paleness of the flesh and organs. The lesions in the mouth have as a rule already healed out.''

The post-mortem appearances of bluetongue in sheep described by Spreull (*loc. cit.*) are fairly exhaustive and it is significant how these correspond with those changes seen in experimental sheep in-fected with virus obtained from cattle. " Frothing at the mouth, swelling of the lips, particularly the upper lip, catarrhal discharge from the nostrils, cyanosis of the buccal mucous membrane, dental pad and inner surface of the lips are raw and excoriated. Sores appear inside the cheeks in the region of the molars and even on the rugae of the hard palate . . . . the tip of the tongue becomes sore . . . . in a few cases the tongue becomes extremely oedematous, quite fills the mouth . . . . there is occasionally an oedema of the lower parts of the face and especially of the skin and tissues under

the jaw, extending through rarely for a short distance down to the under surface of the neck. . . . The rumen often shows inflamed patches more especially affecting the pillars. . . . There may be one or two ulcers at the pylorus. . . . As a rule death occurs from debility and the mouth and other lesions have generally healed by that time . . .''

It is most significant how the changes in natural bluetongue in sheep as described by Theiler and Spreull correspond to those seen by the writers.
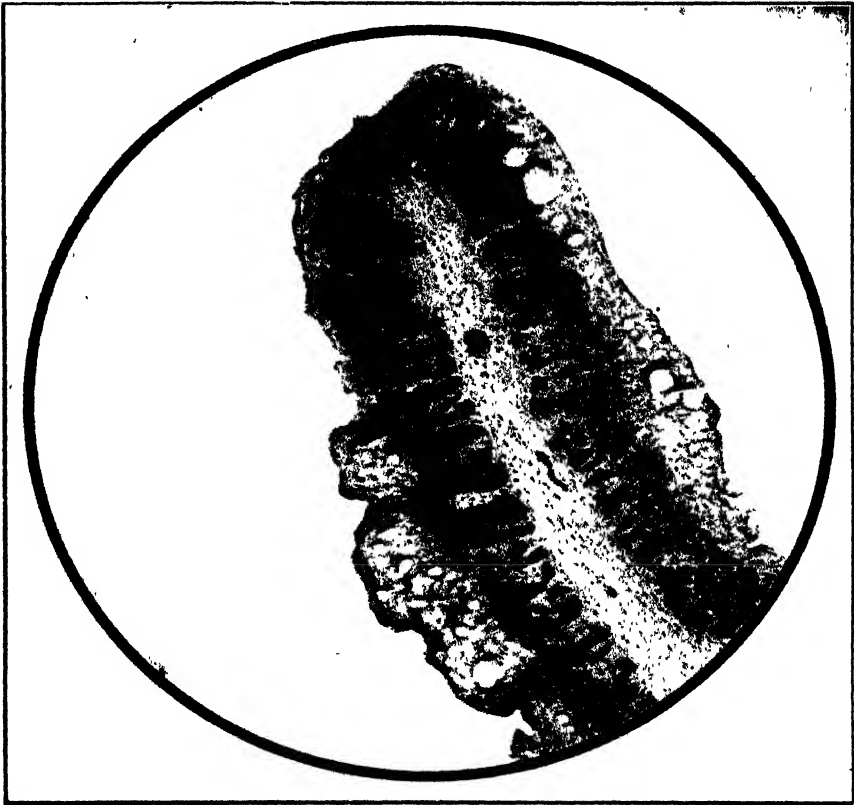
Fig. 33.

Papilla of rumen: Showing "ballooning" of stratified epithelial cells. (Expt. sheep case.)

PATHOGENESIS.

In the Third Progress Report (1928), it is said that the question has often arisen why the lesions of foot and mouth disease should be confined to such very limited areas of the body as the skin, feet, and mucous membrane of the mouth and tongue. . . . It must be supposed that it is only in the superficial layers of the plantar and

palmar skin and of mucous membrane of the tongue that favourable conditions for the multiplication of the virus occur, but what the conditions may be is mysterious. . . . . From the experiments of Maitland it would appear that the position of vesicles is evidently not determined entirely by the structure of the tissues affected . . . . the mild irritation caused by movement and pressure would appear from these experiments to be a necessary condition. . . . With freedom from pressure, the local conditions are less favourable for the propagation of the virus, whether directly implanted or brought by the blood stream. . . .

In bluetongue, especially in the experimental cases where all stages could be carefully watched, it was significant how regularly lesions developed in certain situations, e.g. on the lower lips opposite the incisors, on the dorsal-lateral aspect of the tongue opposite prominent molars, on those parts of the fore-stomachs (muscular pillars, oesophageal groove) and pylorus where function seems more prominent than in other parts; parts of the integument subjected to pressure, etc. In case of the tongue certain parts which were brought into closer contact with the molar teeth became particularly affected (vide Fig. 25).

Microscopical scrutiny of the internal organs in foot and mouth disease was undertaken by the Research Committee, but no lesions were discovered in them . . . . it is concluded that, although the virus established itself in certain restricted areas alone it speedily dies out or succumbs to the natural defences of the body.

Interesting histological observations made in respect of experimental cases of bluetongue bear some resemblance to those referred to on page 10 of the Third Progress Report, viz., '' Where the earliest evidences of a lesion were observed in the epithelial tissue covering the tongues of rabbits and guinea-pigs . . . . a few epithelial cells lost their ordinary fusiform shape and became spherical . . . . their protoplasm had changed its staining property and their nuclei degenerated . . . . in the centre of the lesion the altered cells were broken up and infiltration of the infected area with a few neutrophiles had occurred . . . . both degenerated epithelial cells and infiltrating cells are subsequently disintegrated and the lesion becomes successively a vacuole, a vesicle, a vesicopustule. . . . The latter finally ruptures, leaving an ulcer which heals under a crust. . . .''

In experimental bluetongue the earliest lesions in some of the cases manifested themselves in the epithelial cells in the vicinity of the '' germinal layer '' and even in cells of the basal layer where the cells become spherical, swollen and the cytoplasm shows a diffuse slightly stained hyaline appearance sometimes resembling droplets with a disappearance of the nuclei.

At this stage, as in foot and mouth lesions in rabbits and guinea-pigs, usually no histological changes in the corium could be identified. This stage resembles the condition of '' ballooning '' sometimes also seen in actively growing papillomata of bovines (vide Fig. 31).

At the next stage these cells become disintegrated and their spaces become loosely filled with neutrophiles. These neutrophiles together with the remaining epithelial cells then undergo necrosis, while inflammatory changes also now make their appearance in the underlying part of the corium.

At the next stage portion of the superficial necrotic mass becomes dislodged and leaves a bed composed of necrotic tissue extensively infiltrated with cells and in which the layers of the stratified epithelium are no longer in evidence. Other similar lesions of the mucous membranes did not show typical vesicle formation but transformed immediately into pustule, necrosis and ulceration. Almost identical lesions with " ballooning " of the epithelial cells, infiltration with neutrophiles, necrobioses, etc., were also observed in localized areas on some of the papillae of the rumen.

In foot and mouth disease in rabbits and guinea-pigs characteristic vesicles are only observed on the tongue, whereas extensive histological examination of the liver, brain, spleen, kidney, spinal cord, lung, heart muscle, ovary, testicle, adrenal, parotid of infected guinea-pigs and rabbits failed to disclose any departure from the normal. The Foot and Mouth Disease Research Committee felt justified in concluding that the initial lesions occur in the epithelium of the tongue and that the corium is only secondarily affected and that the histology of the lesion indicates an affinity of the virus for epithelium. The appearance suggest a true intra-epithelial culture.

Rivers (1928) also refers to the effects produced in cells by viruses, especially in respect of the epidermis . . . . " they are subjected to forces not impinging in the same way upon the other cells of the body . . . . they are under the direct influence of a variety of mechanical injuries . . . . whether the virus acts on the surface of the cell or penetrates into it is not known . . . . both the injury to the cell and the multiplication of the virus still have to be explained."

The process of " ballooning " of some of the cells of the stratified epithelial cells has frequently been seen in some of the papillomata of the skin of bovines. The etiology of these multiple papillomata believed to be transmissible has not yet been settled. It certainly does not exclude a virus factor and the possibility of a subsequent immunity, especially in view of their spontaneous disappearance. It will be of great interest to determine whether these changes in the cell cytoplasm observed in bluetongue is of the nature of a degeneration either due to the multiplication and action of the virus or to traumatic influences (e..g pressure of teeth on swollen mucous membranes as a result of oedema or exaggerated function in the vicinity of the pillars of the rumen, etc.

Does the virus multiply and propagate only in those epithelial cells which become previously injured by pressure, etc.?

In spite of a careful examination of sections stained with Giemsa it was not possible to identify anything of the nature of " inclusion bodies ". It is realized that failure to identify such

bodies by the above method does not exclude their presence. On the other hand, in some of the experimental cases this " ballooning " process of the epithelial cells was not seen, but multiple haemorrhages into the epithelial *layers of the mucous membranes.* It is, however, at the present moment not possible to offer any explanation of the actual relation of the epithelial cells of the skin and mucous membranes to the virus, and how this virus multiplies and propagates, especially in those cases where only multiple haemorrhages were observed.

The transudation of fluid at certain stages of the disease into the subcutis of the mandibles, the intermandibular space, the supra-orbital fossa, the lips, the tongue, the upper part of the ventral aspect of the cervical region, the peritracheal and peri-oesophageal connective tissues, lungs, into the body cavities (hydrothorax, hydro-pericard) forms an interesting pathological manifestation in several diseases (e.g. dikkop horsesickness, bluetongue, dunkop horsesick-ness, *cucumis* poisoning). No explanation has yet been given as to why this " transudation " process is so specifically confined to the cranial aspects of the body. It is only in extensive transudation that it may encroach on to upper part of the fore-limbs and on to the region of the shoulder. In dunkop horsesickness it is characteris-tically confined to the lungs, thorax, and submucosa of the respi-ratory tract. In the experimental bluetongue cases the course of the transudation followed more the condition as it occurred in dikkop horsesickness, but in bluetongue morphological changes of the myo-cardium were never of the same degree as those seen in horse-sickness. In fact in bluetongue the myocardium in a number of cases showed no morphological lesions and if this transudation was at all associated with the action of the heart then is was probably of a nervous cheracter.

Quin (1929) in his observations on *cucumis* poisoning speaks of a primary pulmonary oedema, and seems to think that vasomotor disturbances accounting for the oedema seems remote. The poison appears to exert a marked injurious influence on the endothelial lining of the minute vessel walls, so causing a sudden and marked increase in permeability. If we accept a similar explanation for transudation in bluetongue, it will be difficult to co-ordinate that with the extraordinary characteristic localization in the cranial aspects of the body.

THE COURSE AND PROGNOSIS.

In comparatively *mild reactions* the prognosis is favourable. The animals are visibly ill and off their feed for about a week. Recovery is rapid and uneventful.

In *severe affections* a guarded prognosis must be given. There is a rapid loss of condition and marked weakness follows. On account of the extensive changes in the buccal mucosa the appetite remains precarious. Should a dermatitis develop, the recovery is even slower and the injured skin, especially round the mouth, is frequently struck with blowflies.

In several instances the disease terminated in death. In such fatal reactions death usually takes place in the early or acute stages. It would seem that a recovery could be hoped for when once the acute reactions have passed off, but in the later stages cases have been encountered in a moribund condition due to weakness and debility.

Extensive and deep seated necrotic ulcerations have been noted on the tongues of some cases, e.g. in two of the Welgezegend cows. It is very probable that the more or less superficial injury of the mucosa by the virus provides *loci minorum resistentiarum* for the invasion of bacteria, which then contribute towards the formation of the extensive injuries. In one of the Welgezegend cows more than half of the middle portion of the tongue was found at post-mortem examination to be necrotic and gangrenous. It is very probable that, if this animal had been allowed to live the anterior portion of the tongue would have sloughed off.

In some of the experimentally infected calves and sheep similar, although not so extensive, ulcers developed on the tongues. Such lesions took a considerable time to heal and were present a month after the date of infection when all the other lesions had healed.

DIAGNOSIS.

In typical cases the lesions are sufficiently characteristic for a diagnosis to be made. The following changes are important : —

(1) The superficial localized inflammation with necrosis on the lips, conical papillae, dental pad, external nares, muzzle, and ventral aspect of the apical portion of the tongue.

(2) The swelling of the lower portions of the limbs and the marked hyperaemia of the skin particularly in the region of the accessory digits.

(3) The condition of the teats in milking cows; this consists of a necrosis of the epidermis with subsequent scab formation.

(4) In the later stages the dermatitis and subsequent formation of crusts and hard scabs.

For diagnostic purposes the temperature is not of much value. As in sheep, the temperature is elevated during the initial stages of the disease, but as most cases come under observation only in the later stages, i.e. when the mouth lesions, etc., are well developed, a normal or only slightly raised temperature is usually found. Furthermore, under field conditions a temperature diverging only slightly from normal is very difficult to interpret.

The epizootology of this disease in bovines is most important when considering a diagnosis. The disease makes its appearance in late summer and autumn, and generally only a few individuals in

a herd become affected. The disease occurs sporadically. The prevalence of horsesickness and bluetongue in sheep in localities where this disease may make its appearance should be taken into consideration. In cases of doubt, e.g. in some of the atypical cases which may be encountered, the diagnosis may be confirmed by inoculating suspected blood into normal sheep. In this connection Merino sheep, raised under conditions where natural infection is excluded, e.g. the Karroo, are most suitable.

DIFFERENTIAL DIAGNOSIS.

(a) Foot and Mouth Disease.

The following differences are important :—

1. *The Epizootology.*—Foot and mouth disease is a contagious disease and a new outbreak can usually be traced to some source of infection. In the case of bluetongue of cattle outbreaks occur sporadically and there is no evidence that the disease can spread by contact. The latter disease occurs seasonally and simultaneously with horsesickness and bluetongue of sheep.

2. *The lesions.*—The same parts, viz., buccal cavity, feet and udder (teats) are affected in both diseases, there is, however, a distinct difference in the nature of the lesions. In bluetongue the pathological changes are essentially those of a localized inflammation with necrosis of the mucous membrane and the characteristic vesicles of foot and mouth disease are not observed. In bluetongue the lesions show a zone of necrosis bordering on the periphery of the lesion, whereas in foot and mouth disease the frayed irregular remains of the ruptured vesicle can usually be identified. In the later stages the ulcers that are formed in both diseases are somewhat similar and it might be difficult to make a distinction. It should, however, be remembered that the skin lesions are usually observed in the later stages of bluetongue and this is of value in making a differential diagnosis.

Animal reactions.—Foot and mouth disease can be excluded by inoculating sheep. Merino sheep are very susceptible to the virus of bluetongue, and the characteristic reaction of bluetongue is observed.

(b) Snotsiekte.

Here the history of close contact with wildebeeste is a most important factor. Furthermore, well marked and distinguishing pathological changes occur, e.g. the enlargement of the lymphatic glands and the acute catarrhal and pseudo-membranous inflammation of the mucosa of the upper air passages resulting in a profuse muco-catarrhal discharge from the nostrils. Snotsiekte is an acute and nearly always fatal disease and is of the nature of a lymphatic aleucaemia. Very severe eye lesions, keratitis and conjunctivitis, are other important distinguishing features.

According to Mettam (*loc. cit.*) sheep are not susceptible to the virus of snotsiekte.

(c) Malignant Catarrhal Fever.

In his discussion on the differential diagnosis of snotsiekte Mettam (1923) makes the following statement:—

" Malignant catarrhal fever occurs sporadically in South Africa, but to what extent is not known." No reference is given for this information. He refers to this malignant catarrh as a disease accompanied by a high fever and he mentions acute eye and nasal symptoms, rapid emaciation, spreading of the inflammation of the mucous membranes lining the sinuses to that of the horn cores, which often came away in the hand during manipulation. He further states that in many cases of malignant catarrhal fever skin lesions are found in the form of papular exanthema and often in the later stages a profuse scaling of the epidermis. According to Mettam the condition cannot be reproduced in cattle by blood inoculations and he mentions this as a distinguishing feature from snotsiekte. From this description it would appear that this is the same disease that occurs in European countries. Recently Götze (1932) maintained that malignant catarrhal fever and snotsiekte are, if not identical, then very closely related diseases. This conclusion is arrived at by a consideration of the fact that both diseases can be transmitted to susceptible cattle by blood inoculations and because the clinical and pathological-anatomical changes are very similar in both diseases. Götze suspects sheep playing a rôle in malignant catarrhal fever analogous to that which the wildebeest plays in snotsiekte in South Africa.

As far as is known outbreaks of snotsiekte in South Africa have only been observed under circumstances where cattle come into very close contact with wildebeeste. It is not known whether sheep can serve as a reservoir for the virus of snotsiekte in a way similar to that suspected by Götze in the case of outbreaks of malignant catarrhal fever in Germany.

In view, however, of the findings of Götze, further investigations will have to be carried out to establish the exact relationship between snotsiekte and malignant catarrhal fever. It is most important to ascertain whether malignant catarrhal fever does exist as a disease *sui generis* in South Africa. The possibility that the disease, referred to by Mettam as malignant catarrhal fever, might have been the same as this bluetongue of cattle should also be considered.

(d) Sweating Sickness (Sweetsiekte or Nat-kalwersiekte).

This disease has been described by du Toit (1923), who found the condition usually confined to calves only. Later observers, Clark (1933) and A. D. Thomas (in a personal communication), maintain that the occurrence of sweating sickness in older animals is by no means rare. Clark describes a typical case in a three-year-old cow.

The disease apparently occurs enzootically in certain parts of South Africa, such as the Lowveld of Swaziland, Zululand, Natal, in the Bushveld of Northern Transvaal, and Bechuanaland. The aetiology of the condition is still unknown, but du Toit (*loc. cit.*) suggested that a filtrable virus may be the responsible factor.

There are certain resemblances between sweating sickness and certain forms of bluetongue of cattle, in particular the same seasonal incidence and certain clinical conditions, for instance, the hyperaemia and necrosis (ulcers) on the buccal mucosa and the condition of the skin in the later stages. But a condition of sweating has not as yet been observed in cases of bluetongue in cattle, and although older animals may be affected with sweating sickness, the disease is most common in calves, whereas bluetongue has been observed to occur in older cattle.

In view, however, of certain similarities between the two conditions it would be essential to determine whether reactions could be produced in sheep with sweating sickness material and if any relationship exists between the two diseases.

(e) THREE-DAY-STIFFSICKNESS (EPHEMERAL FEVER).

The first symptoms usually observed in bluetongue of cattle is marked with stiffness and lameness. These are usually the only symptoms observed for a day or two by the owners before the mouth lesions become obvious. It has already been suggested (*vide* symptomatology) that cases described as three-day-stiffsickness, especially where they are found in association with typical bluetongue reactions in cattle, may also possibly be atypical forms of the same disease.

In true three-day-stiffsickness a relatively large number of animals (10 to 50 per cent.) are usually affected. Except for the stiffness, slight lachrymation and elevation of temperature no other important symptoms or lesions develop.

According to Theiler (1907) the disease can be transmitted to susceptible cattle by means of blood inoculation. He succeeded in setting up characteristic symptoms of three-day-stiffsickness in a heifer by inoculating her with 20 c.c. defibrinated blood from a natural case.

(f) LAMSIEKTE.

A complete or partial paresis of the tongue is a common symptom observed in lamsiekte. In severe reactions of bluetongue a protrusion of the tongue occurs and in such cases this disease may be confused with lamsiekte. It should be remembered that in lamsiekte no visible lesions are present in the mouth or on the tongue and a complete paralysis of the locomotory system is also evident in practically all cases.

Furthermore, lamsiekte can usually be excluded by carefully considering the epizootology and the various aetiological factors concerned.

(g) RINDERPEST.

In bluetongue the lesions on the lips, dental pad, muzzle and external nares to some extent resemble those seen in the early stages of rinderpest, but the subsequent alarming intestinal disorders of the latter disease do not develop. Furthermore, bluetongue is usually observed in a few individuals in a herd and there is no evidence of the disease spreading by contact, whilst rinderpest is

a highly infectious condition and spreads very rapidly. A further distinguishing characteristic is the very high mortality in rinderpest; bluetongue in comparison is a relatively benign disease.

(h) Stomatitis due to Traumatic or Chemical Agents.

Such factors can usually be excluded by carefully considering the history of each case. Traumatic agents, e.g. the awns of certain grass seeds are usually found *in situ*. It should be remembered that sick animals in South Africa are often subjected to rather drastic and severe treatment and irritating chemical substances such as caustic soda, carbolic acid preparations, etc., are sometimes used. The stomatitis resulting from the use of such substances should be considered when arriving at a diagnosis.

(i) A Form of Panaritium.

This is frequently encountered in South Africa and such an outbreak occurred for instance on a farm Bouwlust in the Koedoesrand area during April, 1933. Apparently this condition is due to contamination of the feet by mud around the drinking water and in hut kraals. In the outbreak at Bouwlust the condition was apparently exaggerated by the fact that the cattle had to be driven over a long distance to the Magalakwin river for watering purposes. The hind limbs in this case were most affected and no mouth lesions were observed.

From the present South African point of view the above conditions are, no doubt, the most important which should be taken into consideration in making a differential diagnosis. However, since this disease in some clinical respects resembles foot and mouth disease, and, furthermore, since it first came into prominence during a campaign against the latter in South Africa, the differential diagnosis would be incomplete without including certain other conditions which are usually brought under review in a consideration of the diagnosis of foot and mouth disease.

Eaton (1933) has recently presented an excellent review of conditions of the mouth and feet of cloven-footed animals in a discussion of the diagnosis of foot and mouth disease. The following are some of the conditions included in his long list :—

1. *Vesicular stomatitis.*—Here only mouth lesions develop and consist of vesicles, usually seen only on the tongue, but occasionally on the gums. Important differential characteristics are the absence of feet lesions and no locomotory disturbances.

2. *A non-specific catarrhal and ulcerative stomatitis.*—These conditions are usually noted in animals which are poor and unthrifty. In the case of the catarrhal form heaped-up epithelial deposits are to be observed on the tongue, hard palate, and inside the upper and lower lips. In the ulcerated form the ulcers heal slowly.

3. *Calf diphtheria.*—Confined to calves. The mouth lesions area caused by the *bacillus necrophorus* after a preliminary injury to the buccal mucosa such as is caused by teething. The yellow necrotic patches are to be seen on the dorsum of the tongue and inside the cheeks. Large sloughing of the affected parts often follows and death is a common sequel.

4. *Pustular stomatitis.*—According to Eaton this condition is met with by itself or with an accompanying dermatitis in young cattle, and also in sheep. The lesions are found on the inside and outside of the lips. Pus is discharged which forms crusts on the outside of the lips.

Under this heading the well-known South African disease Vuilbek (*Ecthyma contagiosum*) of sheep and goats can be conveniently considered. Vuilbek is a benign disease when compared with bluetongue of sheep and foot lesions are absent. Ordinarily very little difficulty is encountered in distinguishing it from bluetongue in sheep, but when lesions occur on the inside of the lips and cheeks it might be necessary to resort to biological tests.

Eaton discusses conditions such as Dirty tongue (Armagh disease), Actinomycosis of the tongue, Actinobacillosis of the dental pad, traumatic and dental injuries of the buccal mucosa, which could from a pathological-anatomical consideration be confused with foot and mouth disease. He also draws attention to choking, lactation tetany, disturbances of the salivary glands, etc., where salivation is a conspicuous symptom, and may thus also be confused with foot and mouth disease.

SUMMARY AND CONCLUSIONS.

During the autumn of 1933 an undescribed disease referred to as pseudo-foot and mouth disease, made its appearance in cattle in herds scattered over an extensive area of South Africa. From evidence obtained it would appear as if this disease is not a " *new* " condition, *but that it has been observed* for some considerable time. Unfortunately no serious notice was taken of it and it thus escaped the attention of veterinarians until the beginning of 1933 when all possible veterinary resources were organized to deal with a possible spread of foot and mouth disease throughout the Union.

In practically every case where this so-called pseudo-foot and mouth disease broke out the owners considered it either as foot and mouth disease, or, at any rate, very suspicious. Since lesions appeared on the buccal mucosa, the feet and on the udders, veterinarians experienced considerable difficulties in definitely excluding foot and mouth disease. It, therefore, became essential to undertake experimental investigations in order to ascertain the nature of this condition.

In the experiments undertaken it was possible to indicate the presence of the well-known virus causing bluetongue of sheep in the blood of most of the cattle suffering from this " pseudo-foot and mouth disease." Very characteristic reactions of bluetongue developed in the experimentally infected sheep, and, furthermore, the specificity of the virus was established by immunological tests.

It was also shown that calves, although less susceptible than sheep, undergo a definite reaction when infected with virus originally obtained from cattle affected with this disease.

In the discussion of the pathogenesis of bluetongue reference was made to the significance of the lesions, especially in relation

to the stratified epithelial cells of some of the mucous membranes, and the question of transudation into the connective tissue, especially of the subcutis of the cranial aspect of the body.

From an actual economical aspect the occurrence of bluetongue in cattle is apparently of minor significance, for in most of the outbreaks which were investigated only a small percentage of animals were affected, and these cases usually made an eventful recovery. It is from a differential diagnostic point of view, especially in connection with foot and mouth disease that the occurrence of bluetongue is of very great importance.

The knowledge that cattle are susceptible to the virus of bluetongue may perhaps throw further light on certain cattle diseases of South Africa where the aetiology is still somewhat obscure. This is especially the case in connection with sweating sickness of calves, the alleged occurrence of malignant catarrhal fever, and some of the so-called cases of three-day-stiffsickness.

REFERENCES.

CLARK, R. (1933). Observations on Sweating Sickness in Northern Zululand. *Jnl. of S.A. Vet. Med. Ass.*, Vol. 4, No. 1.

DU TOIT, P. J. (1923). Sweating Sickness in Calves. *9th and 10th Rept., Dir. Vet. Educ. and Res.*, U. of S.A.

DU TOIT, P. J. (1929). Studies on the Virus of Bluetongue. *15th Rept., Dir. Vet. Serv.*, U. of S.A.

DU TOIT, P. J. (1933). Pseudo-Foot and Mouth Disease and the True Form. *Farmer's Weekly*, Vol. 45, No. 1249.

EATON, G. (1933). The Differential Diagnosis of Diseased Conditions and Abnormalities of the Mouth and Feet of Cattle, Sheep, and Swine, with particular reference to Foot and Mouth Disease. *The Vet. Rec.*, Vol. 13, No. 35.

GÖTZE, R. (1932). Bösargtige Katarrhall fieber. *Berlin Tierarzliche Wochenschrift*, Vol. 48, No. 53.

HENNING, M. W. (1932). Animal Diseases in South Africa (Central News Agency), pp. 503-515.

KEPPEL, J. J. G., AND ROBINSON, E. M. (1932). An Outbreak of Ulcerative Stomatitis in Cattle. *Jnl. of S.A. Vet. Med. Assn.*, Vol. 3, No. 4, p. 176.

METTAM, R. W. M. (1923). Snotsiekte in Cattle. *9th and 10th Rept., Dir. of Vet. Educ. and Res.*, U. of S.A.

NEITZ, W. O. (1933). The Blesbuck (*Damaliscus albifrons*) as a Carrier of Heartwater and Bluetongue. *Jnl. S.A.V.M.A.*, Vol. 4, No. 1.

QUIN, J. I. (1929). The Toxic Properties of *Cucumis myriocarpus* and *C. africanus*. *15th Annual Report of the Dir. Vet. Serv.*, Onderstepoort, Pretoria, pp. 769-777.

RIVERS, T. M. (1928). Filterable Viruses. The Williams & Wilkens Co., Baltimore.

SPREULL, J. (1905). Malarial Catarrhal Fever (Bluetongue) of Sheep in South Africa. *Jnl. Comp. Path. and Therap*, Vol. 18, p. 321.

THEILER, A. (1905). Blutongue in Sheep. *Ann. Rept. (1904-1905), Dir. of Agric., Transvaal*, p. 110.

THEILER, A. (1918). Paralysis of the Oesophagus in the Horse as a Sequel of Horsesickness. *7th and 8th Rept., Dir. of Vet. Res.*

Third Progress Report of the Foot and Mouth Disease Research Committee (1928), Ministry of Agriculture and Fisheries.

APPENDIX A.

TRANSMISSION EXPERIMENTS.

INTRODUCTION.

Transmission experiments were undertaken with material obtained from various outbreaks. These experiments were commenced on the farm Welgezegend and continued later at Onderstepoort. It is convenient to give the results under various headings indicating the source of the infective material:—

(1) Experiments with *Welgezegend* cattle virus:—

    (*a*) Series with a strain recovered from a sick cow (case 1).

      (*b*) Series with a strain recovered from a calf which was "infected" intranasally with a mixture of blood, urine, milk, and emulsified necrotic tissue from three sick cows (cases 1, 2, and 3).

      (*c*) Series undertaken with a strain recovered from an ox which developed a fairly mild reaction and later recovered.

(2) Experiments with *Kromdraai* cattle virus.

(3) Experiments with *Darling* cattle virus.

(4) Experiments with *Elandslaagte* cattle virus.

(5) Experiments with *Novo* cattle virus.

(6) Experiments with *Montague* cattle virus.

(7) Experiments with *Onlangs* cattle virus.

(8) Experiments with *Swartland* cattle virus.

(9) Experiments with a strain of bluetongue virus recovered from a sheep at *Novo*.

(10) Miscellaneous experiments.

Each chapter is arranged in the following way:—

    A. A description of the outbreak.

    B. Table indicating the transmission experiments to which the various strains were subjected.

    C. Tabulation of experimental results.

        NOTE.—The reactions which were observed in the calves are fully described, but as far as those in the sheep are concerned only the nature of the reaction is mentioned. The reactions were typical and are fully described in first part of the paper (*vide* symptomatology). Any complications are specially mentioned.

    D. Summary of the results:—

      (1) Calves 1-14 were used in the Welgezegend experiments.

      (2) Sheep 1-19 were used in the Welgezegend experiments.

      (3) All the remaining calves and sheep were kept under laboratory conditions at Onderstepoort.

      (4) The sheep used in the Onderstepoort experiments were all originally from the Karroo, i.e. reared under conditions where bluetongue does not usually occur.

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(1) Experiments with Welgezegend Cattle Strains.

History of the Outbreak.

A total number of 308 head of cattle were kept on the particular portion of the farm Welgezegend where this outbreak occurred. The cattle were in low condition on account of a drought, but they were free of specific disease. On the 10th March three cows became ill. They were noticed to be slightly stiff and lame. No serious attention was given to the condition and a vague diagnosis of " gallsickness " was attempted. The animals were accordingly drenched. Three days later, however, marked salivation was observed and in one case the tongue was protruding from the mouth. The mouths were now examined and alarming " sores " discovered. Foot and mouth disease was immediately suspected and the disease reported to the veterinary officer.

Condition of the Cows on 15th March.

Case No. 1.—The animal, a young grade Friesland cow, was found lying down. There was marked salivation and the temperature 102·6°. The animal showed no inclination to feed and appeared very depressed.

The skin on the plantar region of the limbs, up to the fetlocks, appeared red and swollen. The epidermis in the interdigital spaces appeared excoriated and the underlying tissues necrosed and moist. Loose and partially detached epidermis was still adherent in parts. On rising a marked lameness in all four limbs was observed.

The udder showed a dermatitis in the stadium squamosa with a marked red discoloration of the teats.

A moderate amount of salivation was observed and in the mouth an elongated superficial necrosed area was present between the, upper lip and dental pad. Similar lesions were present on the border of the lower lip. On the left side of the tongue there was a large area, extending from the middle third to the posterior third which appeared necrosed. This area was covered with a greyish-yellow material. The borders of this lesion were very red. Extensive necrotic lesions were noted on the dental pad. These were also covered with yellowish material. Superficial necrosis and ulceration was also noted along the borders of the nose. The muzzle was dry and encrustated.

Case No. 2: A Young Grade Friesland Cow.—The condition of this cow was similar to case 1, with the following exceptions:—

The necrosis on the tongue was confined to the ventral aspect of the apex. The lips and dental pad were more extensively involved. The tongue appeared swollen and was hanging from the mouth. The lesions in the interdigital space were not so marked. Salivation was much more profuse.

Case No. 3: A Young Grade Friesland Cow.—This was a comparatively mild case. No feet lesions were to be found. The udder was only slightly affected and the mouth lesions not so marked. An elongated necrosed area was present in the space between the upper lip and dental pad and similar lesions on the dental pad. A few irregular and fairly small necrosed lesions were noted on the lateral aspect of the apex of the tongue. Small irregular necrosed areas were present on the nose.

The above cows were destroyed. Post-mortem examinations were undertaken and are described in Appendix B.

Subsequently (17th April) two further cases were discovered on this farm. The affected animals were in both cases mature oxen. The lesions were comparatively mild and necrotic lesions were noticed on the dental pad, the upper and lower lips, and the ventral surface of the apex of the tongue. The lower parts of the limbs appeared swollen and the skin on the plantar region of digit was red. Fairly high temperatures (more than 105° F.) were recorded on several occasions. These oxen ultimately recovered.

A series of three experimental investigations were undertaken with material from these cases:—

1 (a) with a strain recovered from a sick cow (case 2, above).

1 (b) with a strain recovered from a calf which was " infected " intranasally with material from the cows (cases 1, 2, 3).

1 (c) with a strain recovered from one of the affected oxen.

EXPERIMENTS 1 (a).

Table to indicate the transmissions undertaken with virus from affected cow (case 2).
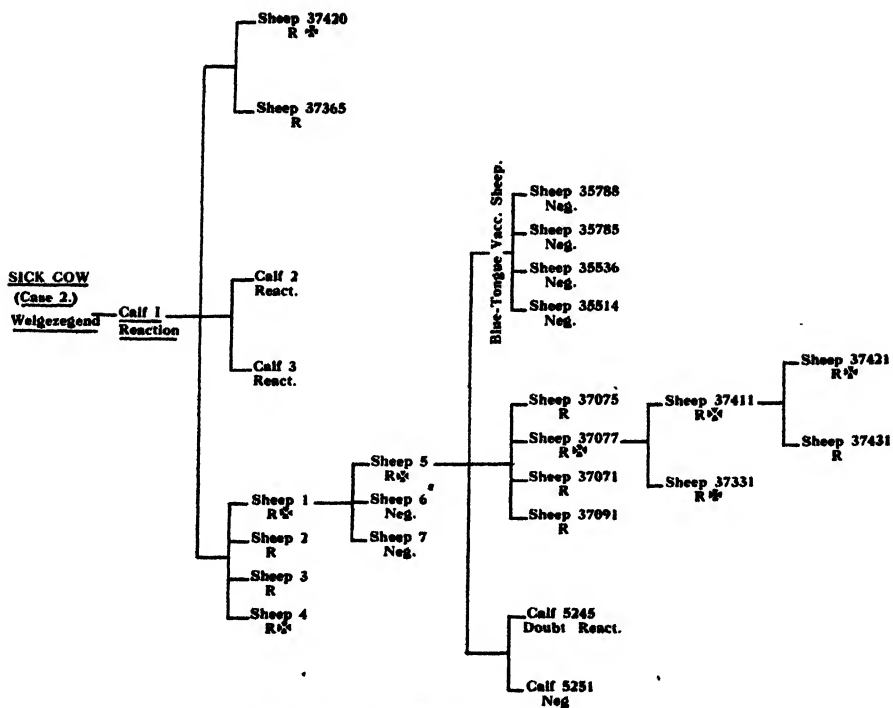
Note : R—Blue Tongue Reaction,
R✠—Blue-Tongue Reaction terminating in Death.'

Experiments with Welgezegend Virus recovered from sick cow (case 2).

Table 1 (a).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Calf No. 1....	*17.3.33*, 20 c.c. fresh blood ex cow case (ii), intravenously	The temperature commenced rising on the second day and reached 106° on the fourth day. After that it gradually fell and could be considered normal on the 12th day. On the third day small superficial ulcers ($\pm$ ·5 cm. in diameter) were seen on the mucous membrane of upper and lower lips; these ulcers commenced healing on the 14th day and gradually disappeared. The animal was killed on the 27th day, and a fairly large ulcerative area was found on the lateral aspect of the fixed portion of the tongue.
Calf No. 2....	*22.3.33*, 25 c.c. fresh blood ex calf No. 1, intravenously *Note.*—Blood collected on 5th day	Slight rise in temperature after third day and reached 104·4° on 6th. Temperature normal on 8th day. On the 6th day superficial ulcers were noted on the upper and lower lips, similar in appearance to those seen in calf No. 1. After the 16th day these areas showed distinct signs of healing and were completely healed on the 21st day, when the animal was destroyed. No unusual changes were present at post-mortem examination.
Calf No. 3....	,, ,,	Distinct temperature reaction beginning on 4th day. Acme (106°) on 6th day. On this day the animal appeared ill, it refused its milk and was lying down. The animal commenced feeding on the 11th day. A marked reddening of the mucous membrane of the mouth was noted on the 9th day. Ulceration of the gums especially along the incisors was subsequently observed. The gingivitis persisted to the 15th day, on this day a muco-purulent discharge developed from the eyes and nose and a slight excoriation of the interdigital spaces was observed. Destroyed on the 21st day.
Sheep No. 1..	*23.3.33*, 5 c.c. fresh blood ex calf No. 1, subcutaneously	Fatal peracute reaction. I.P.*—3 days. Died on 9th day.
Sheep No. 2..	,, ,,	Acute reaction and recovered. I.P.—5 days.
Sheep No. 3..	,, ,,	Acute reaction and recovered. I.P.—3 days.
Sheep No. 4..	,, ,,	Fatal peracute reaction. I.P.—very short about 24 hours. Died on 7th day.
Sheep No. 37365	*26.4.33*, 5 c.c. blood ex calf No. 1, intravenously *Note.*—Blood collected on 6th day, preserved in O.G.C. and stored for 45 days at ord. room temperat.	Subacute reaction, complicated with peritonitis (perforation of rectum). I.P.—6 days. Died on 14th day.
Sheep No. 37420	,, ,,	Fatal acute reaction. I.P.—6 days. *In extremis* on 13th day and destroyed.

* I.P.—Incubation period.

Table 1 (*a*)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Sheep No. 5..	*30.3.33*, 5 c.c. fresh blood ex Sheep No. 1 subcut. *Note.*—Blood collected on 7th day	Acute reaction. In the later stages marked oedema of the lungs developed (c.f. dunkop horsesickness). I.P.—6 days. Died on 12th day.
Sheep No. 6..	*30.3.33*, 5 c.c. fresh blood ex sheep No. 1, injected intra-nasally *Note.*—Blood collected on 7th day	No reaction. (This experiment was undertaken to determine whether sheep could be infected by an intranasal injection of virulent blood.)
Sheep No. 7..	,,　　,,	No reaction.
Sheep No. 35788 (B.T.V. sheep)	*19.4.33*, 1 c.c. blood ex sheep No. 5 *Note.*—Blood collected on 9th day, preserved in O.G.C. and stored at ordinary room temp. for 41 days	,,　　,,
Sheep No. 35785 (B.T. Vacc. sheep)	,,　　,,	,,　　,,
Sheep No. 35536 (B.T. Vacc. sheep)	,,　　,,	,,　　,,
Sheep No. 35514 (B.T.V. sheep)	,,　　,,	,,　　,,
Sheep No. 37075	*22.3.33*, 25 c.c. fresh blood ex calf No. 1, intravenously *Note.*—Blood collected on 5th day	Mild acute reaction and recovery. I.P.—7 days. *Immunity Test.*—22nd day, 1 c.c. blood ex natural bluetongue reaction in a sheep at Novo. No reaction developed. *Note.*—Refer to experiment 9 for the tests to ascertain the virulence of this virus.
Sheep No. 37071	,,　　,,	Subacute reaction and recovery. I.P.—5 days. *Immunity Test.*—As with sheep No. 37073—no reaction developed.
Sheep No. 37091	,,　　,,	Mild acute reaction. I.P.—6 days. *Immunity Test.*—As with sheep No. 37075. Definite positive reaction. The following symptoms noted:—I.P.—5 days, high temperature, muco-haemorrhagic discharge nostrils, hyperaemia and excoriations of mucous membrane, nose, bucal cavity, and later coronitis.
Sheep No. 37077	,,　　,,	Fatal acute reaction. I.P.—2 days. Died on 12th day.

Table 1 (*a*)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Calf No. 5245	*19.4.33*, 5 c.c. of sample of blood used in sheep No. 35788, etc.	Slight rise of temperature reaching 103·6° on 6th day No other lesions. Very doubtful reaction. *Immunity Test.*—On 4.5.33, i.e. 16th day, 5 c.c. fresh blood ex sheep No. 37265 (*vide* expt. Ib.) intravenously. No reaction observed.
Calf No. 5251	,, ,,	No reaction. *Immunity Test.*—As with calf No. 5245. No reaction observed.
Sheep No. 37331	*26.4.33*, 5 c.c. fresh blood ex sheep No. 37077 intravenously *Note.*—Blood collected on 8th day	Fatal peracute reaction. I.P.—36 hours. Died on 6th day.
Sheep No. 37411	,, ,,	Fatal acute reaction. I.P.—4 days. Died on 14th day.
Sheep No. 37421	*1.5.33*, 5 c.c. fresh blood ex sheep No. 37331, intravenously *Note.*—Blood collected on 6th day	Fatal acute reaction. I.P.—3 days. Died on 15th day.
Sheep No. 37431	,, ,,	Subacute reaction and recovery. I.P.—3 days. *Immunity Test.*—1 c.c. virulent blood ex sheep Novo. No reaction.

Summary of Results.

(1) Sheep experiments:—

    (*a*) Fifteen normal sheep were inoculated intravenously or subcutaneously with infective material from this source and all reacted. Eight, or more than 50 per cent., died. Deaths occurred from the 6th to 16th day after infection. The incubation period varied from as short as 24 hours to 6 days.

    (*b*) Two sheep " infected " intranasally with 5 c.c. active blood did not react.

    (*c*) *Immunity*:—

        (i) The immunity of four sheep which had recovered from this strain of cattle virus was tested with a virulent strain of bluetongue virus recovered from a sheep. Three proved to be completely immune, whilst one again reacted.

        (ii) Four bluetongue vaccine sheep proved to be completely immune to virus originally recovered from the cow.

(2) Calf experiments:—

    Five calves were inoculated with virus containing material and four developed definite reactions. The incubation periods and the temperature reactions resembled those of bluetongue in sheep, but the mouth lesions were not as characteristic. In most cases excoriations with subsequent slight ulceration appeared on the mucous membrane of the upper and lower lips.

EXPERIMENTS 1 (b).

Table to indicate the transmissions undertaken with virus recovered from affected cows (cases 1, 2, 3).

SICK COWS

Cases i ii iii

20 c.c.s Mixture of Blood, Urine, Milk and Emulsified Necrotic Tissue intranasally into.

Calf 4 — Neg.
Calf 5 — React.
Calf 6 — React.
Calf 7 — React.
Calf 8 — React.

Calf 9 — Reaction
Calf 10 — Reaction

Sheep 8 R ✖
Sheep 9 R ✖
Sheep 10 R
Sheep 11 R

Calf 11 — React.
Calf 12 — React.
Calf 13 — React.
Calf 14 — React.

Sheep 37265 R ✖
Sheep 37298 R ✖

Calf 5206 — React.
Calf 5263 — React.
Calf 5245 — Neg.
Calf 5251 — Neg.
Calf 5204 — Slight Temp.
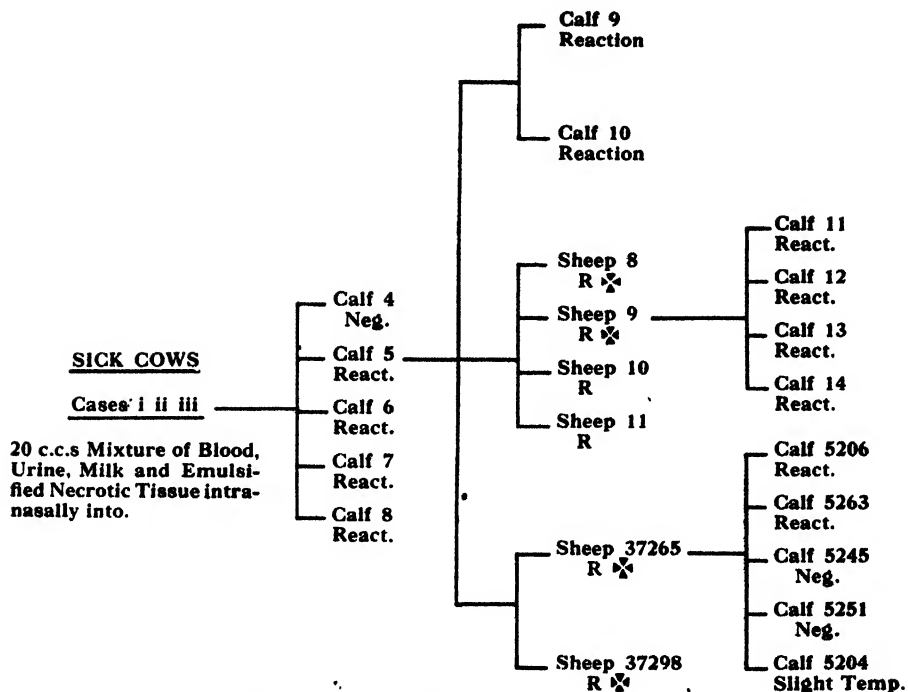
Note : R = Blue-Tongue Reaction.
R.✖ = Blue-Tongue Reaction terminating in, death.

Table 1 (b).
Experiments with Welgezegend Virus.—(b) *From material of sick cows*
(cases I, II, III.)

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Calf No. 4..	17.3.33, 10 c.c. mixture of blood urine, milk, and emulsified necrotic tissues, collected at post-mortem of of cases I, II, and III, intranasally *	No reaction.
Calf No. 5....	17 3.33, infected as calf No. 5	Irregular temperature, which rose to 105° on 5th day. On the 3rd day small superficial ulcers appeared on the mucosa of the upper and lower lips. On the 4th day these ulcers were larger and the lips appeared slightly swollen. On the 10th animal appeared ill and mucosa of mouth reddened, the lesions still present on lips.
Calf No. 6....	,, ,,	No apparent rise in temperature. On the 3rd day superficial ulcers noted on the lower lip opposite lateral incisor teeth. On the 12th day marked dyspnoea noted. Animal destroyed on 13th day, and a fairly marked oedema of lungs found.
Calf No. 7....	17.3.33, 20 c.c. mixture of blood and urine collected from cases I, II, and III, intranasally	Steady rise in temperature from 5th day and 106·2° reach on 13th day. On the 8th day animal appeared to be ill and 12th day a muco-purulent discharge from the nose was observed. Superficial ulcers noted on the lips and later (20th day) a fairly extensive ulcer was noted on the dorsum of posterior part of tongue.
Calf No. 8....	17.3.33, infected as calf No. 6	No reaction.
Calf No. 9....	22.3.33, 25 c.c. fresh blood from calf No. 5, intravenously. (Blood taken on 6th day.)	Rise of temperature on 10th day, reaching 104·6° on 12th day. Temperature remained at this level until 18th day and then gradually fell. On the 5th day superficial ulcers appeared on upper lip and 8th day on lower lip. On 16th day slight excoriation was noticed in the interdigital spaces.
Calf No. 10...	22.3.33, inoculated as calf No. 9	On 5th temperature rose and reached 106° on 7th day. On the 3rd day small superficial ulcers noted on the mucosa of lower lip opposite lateral incisors. On the 10th day marked lachrymation noted and animal coughed frequently. On the 14th day muco-purulent discharge from nose. On 16th day discharge from eyes and nose marked. Lesions on lip still present and a fairly large necrotic ulcer on lateral aspect of tongue. 17th day animal ill lying down and marked dyspnoea present. On the 18th day animal destroyed and at post-mortem examination extensive and fairly deep-seated necrotic lesions found on lateral aspect of posterior portion of tongue and in the larynx.

* The injection of this material into the nasal cavities was primarily undertaken to exclude foot and mouth disease. During the outbreak of foot and mouth disease in Southern Rhodesia, Bevan found that the disease could be easily transmitted by injecting virus containing material into the nostrils of susceptible animals.

Table 1 (b)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Sheep No. 8..	22.3.33, 10 c.c. fresh blood ex calf No. 5, intravenously *Note.*—Blood collected on 6th day	Fatal acute reaction. I.P.—6 days. Died on 13th day.
Sheep No. 9..	,,　　　　,,	Fatal peracute reaction. I.P.—4 days. Died on 7th day.
Sheep No. 10	,,　　　　,,	Acute reaction and recovery. I.P.—5 days.
Sheep No. 11	,,　　　　,,	Acute reaction and recovery. I.P.—3 days. In the later stages deep-seated necrotic ulcers on dorsal and lateral aspects of tongue.
Sheep No. 37298	26.4.33, 5 c.c. blood ex calf No. 5, intravenously *Note.*—Blood collected on 5th day, preserved in O.G.C. and stored at O.R.T. for 29 days	Fatal subacute reaction. I.P.—5 days. Moribund on 20th day and destroyed.
Sheep No. 37265	,,　　　　,,	Peracute reaction. I.P.—5 days. Died on 9th day.
Calf No. 11...	1.4.33, 5 c.c. fresh blood ex sheep No. 9, intravenously *Note.*—Blood collected on 10th day	No definite rise in temperature. On the 6th day appeared ill and would not rise. On the 10th day excoriations noted on the inner surface of lips opposite lateral incisors.
Calf No. 12...	,,　　　　,,	Irregular temperature. 104·3° F. on 3rd day and 105·3° on 9th day. On the 5th day muzzle appeared dry and a watery discharge from nose and eyes. Calf appeared ill and lay down frequently.
Calf No. 13...	1.4.33, inoculated as calf No. 11	Distinct rise in temperature which commenced on 3rd day. 104·6° on 9th day. On 6th day small superficial excoriations noted on the inner aspect of lower lips. These superficial ulcers increased in size. Animal destroyed on 13.4.33.
Calf No. 14...	1.4.33, inoculated as calf No. 11	After third day temperature rose steadily and reached 104·2° on 9th day. Except for slight watery discharge from eyes on 6th day no other lesions or symptoms developed.
Calf No. 5206	4.5.33, 10 c.c. fresh blood ex sheep No. 37265 intravenously (blood collected on 9th day)	Slight rise in temperature on the 10th. The mucosa of bucal cavity appeared reddened and small superficial ulcerative areas (± ·5 cm. in length) appeared on the mucosa of lower lip opposite the lateral incisors. The hyperaemia and lesions persisted for 4 days after which they disappeared. *Note.*—This animal was inoculated with 5 c.c. of blood on 19.3.33 from Onlangs, case I—the blood of this animal proved to be avirulent [*vide* experiments 7 (a)].

Table 1 (*b*)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Calf No. 5263	*4.5.33*, inoculated as calf No. 5206	Slight rise in temperature on third day and remained more than 103° for 5 days. On the 6th day the mucosa of mouth appeared slightly red. On the next day the following changes were observed: the lower lip appeared slightly swollen and the skin red; the muzzle definitely red (white-faced animal); the apical portion of tongue reddened a few of the papillae dark red; the borders of the lip appeared reddened and some of the conical papillae (especially at the angle of the mouth) enlarged, their tips red and in a few the tips greyish-yellow; excoriations of the mucosa was noted on ventral aspect of apical portion of tongue and on the inner surface of the lower lip; in the vicinity of these excoriations numerous petechiae observed. Similar petechiae observed in the groove between the dental pad and upper lip. Lachrymation was observed from both eyes. After 5 days the lesions gradually disappeared. *Note.*—On 19.3.33 this animal was also inoculated with the avirulent blood from case I, at Onlangs.
Calf No. 5245	*4.5.33*, inoculated as calf No. 5206	No reaction. *Note.*—On 19.3.33 this animal inoculated with 5 c.c blood ex sheep No. 5 [*vide* experiment 1 (*a*)], and although no definite reaction was observed, it is more than likely that this animal was immunised as the blood was virulent.
Calf No. 5251	„ „	No reaction. *Note.*—This animal was inoculated on 19.3.33 as calf No. 5245.
Calf No. 5204	„ „	Temperature rose to 103·4° on third day and remained at this level for 6 days. No other lesions developed.

Summary of Results.

I. *Calves.*—Sixteen calves were used in this series of experiments, with the following results:—

(*a*) Four out of the five calves which had received an intranasal injection of mixture of blood, urine, emulsified necrotic tissue, and milk from the three sick cows reacted. The nature of the reactions were similar in each case and were like those noted in calves which reacted to a subcutaneous or intravenous inoculation of blood containing virus. This method of infection was subsequently tested out in sheep, and it was possible to set up a reaction by an intranasal injection of virus containing material [*vide* experiment 10 (*c*)].

(*b*) Blood collected from one of the above calves (No. 5) proved to be virulent since reactions were provoked in sheep and calves.

(*c*) Calves 11, 12, 13, and 14 reacted to the virus after it had been passed through a sheep. The observations, however, were curtailed, as the experiments at Welgezegend had to be stopped.

(*d*) Calves 5206 and 5263 reacted after inoculation with blood from sheep 37265. The lesions observed in the buccal and nasal cavities of calf 5263 were like those seen in typical natural cattle cases, but of a comparatively mild character. Calf 5204 developed a slight temperature.

II. *Sheep.*—Six sheep were inoculated with this virus, and in all typical blue-tongue reactions were observed. Four of these animals died.

III. *Immunity Tests.*—Calves 5245 and 5251, which were previously used in experiments 1 (*a*) did not react.

EXPERIMENTS 1 (*c*).

Table to indicate the transmission experiments undertaken with virus recovered from an affected ox.

N.B.— R=Reaction.
R✠=Reaction terminating in death.
Neg.=No Reaction.

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Table 1 (*c*).

Experiments with Welgezegend Virus.—From an affected ox.

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Sheep No. 12	22.3.33, 2·5 c.c. fresh blood ex sick ox, subcutaneously	Fatal acute reaction. I.P.—3 days. Died on 13th day.
Sheep No. 13	,, ,,	Acute reaction and recovery. I.P.—3 days.
Sheep No. 14	,, ,,	,, ,, ,, ,,
Sheep No. 15	,, ,,	Fatal peracute reaction. I.P.—3 days. Died on 8th day.
Sheep No. 37328	26.4.33, 2 5 c.c. partially decomposed blood ex sick ox, intraven. *Note.*—Blood kept for 35 days at O.R.T. and no preservative added	Fatal subacute reaction. I.P.—3 days. Died on 16th day. In the last stages extensive subcutaneous oedema noted (c.f. dikkop horsesickness).
Sheep No. 37293	,, ,,	Fatal peracute reaction. I.P.—2 days. Died on 7th day.
Sheep No. 16	30.3.33, 5 c.c. fresh blood ex sheep No. 15, subcutaneously	Fatal acute reaction. I.P.—5 days. Died on 12th day. In the last stages marked discharge of serous material and froth through nostrils (c.f. dunkop horsesickness).
Sheep No. 37267	10.5.33, 1 c.c. blood ex sheep No. 37328, subcut. *Note.*—Blood collected on 9th day, preserved in O.G.C. and kept in cold storage	Fatal acute reaction. I.P.—5 days. Died on 12th day.
Sheep No. 37384	,, ,,	Fairly mild acute reaction and recovery. I.P.—7 days. *Immunity Test.*—27th day, 1 c.c. virulent blood ex sheep Novo.—No reaction.
Sheep No. 35512 (B. T.V. sheep)	2.5.33, 5 c.c. fresh defibrinated blood ex sheep No. 37293, subcut. *Note.*—Blood collected on 9th day	No reaction.
Sheep No. 35779 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 35797 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 35774 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 35551 (B. T.V. sheep)	,, ,,	Mild acute reaction and recovery. I.P.—4 days. Slight swelling and injection of buccal mucosa only symptoms noted.

Table 1 (c)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Sheep No. 37207	2.5.33, 5 c.c. fresh defibrinated blood ex sheep No. 37293, subcut. *Note.*—Blood collected on 9th day	Fatal acute reaction. I.P.—6 days. Died on 12th day.
Sheep No. 37297	„ „	Fatal acute reaction. I.P.—6 days. Died on 14th day.
Sheep No. 37274	„ „	Subacute reaction and recovery. I.P.—3 days.
Sheep No. 37464	„ „	„ „ „ „
Sheep No. 37367	„ „	„ „ „ „
Sheep No. 37280	26.4.33, 5 c.c. blood ex sheep No. 16 *Note.*—Blood collected on 8th day, preserved in O.G.C. and kept at O.R. temperature for 18 days	Acute reaction. I.P.—7 days. After the 13th day temperature rose again and complication of bronchopneumonia developed. Animal ultimately died from this. The broncho-pneumonia apparently a condition *per se.*
Sheep No. 37373	„ „	Subacute reaction and recovery. I.P.—6 days. *Immunity Test.*—On 27th day 1 c.c. virulent bluetongue blood ex sheep Novo—No reaction developed.
Calf No. 5292	17.5.33, 20 c.c. fresh blood ex sheep No. 37267, intravenously	*On the 3rd day temperature rose to 105·2° C., and after that an irregular temperature was observed with increases to 104° C. On the 4th day excoriated areas were noticed on the mucosa of the upper and lower lips and localised hyperaemic areas noted on the rugae of the hard palate. On the 6th day lesions on lips showed definite signs of healing and were practically completely healed on the 12th day.
Calf No. 5308	„ „	Irregular temperature no definite elevation. On the 3rd day a slight amount of foam was noticed in the mouth, and on the left side of the lower lip a small area was noted, the mucous membrane of which appeared necrotic, this area was ·5 by ·3 cm. in size. Several hyperaemic areas were present on the rugae of the hard palate. Small excoriations were seen on the mucous membrance of the nostril close to the borders. On the 5th day several new superficial necrotic lesions were observed on the mucous membrane of the upper and lower lips and the hyperaemic areas on the hard palate appeared more extensive. By the 12th day the lesions appeared practically healed out.

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Table 1 (c)—(contd.).

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.
Calf No. 5407	*17.5.33*, 20 c.c. fresh blopd ex sheep No. 37267, intravenously	Rise of temperature on 3rd day and remained at 104° for 24 hours. On the 3rd day the lower lip appeared distinctly swollen and protruded slightly. The gums of the incisors appeared reddened and on the inside of the lower lip two fairly extensive areas (1 5 by 2 cm.) were present, the borders of these lesions had a distinct red zone (*vide* plate (xvii). Similar lesions appeared on other sites of the lower lip and also on the upper lip during the course of the next few days. Hyperaemic areas were also observed on the hard palate. On the 12th day the lesions were practically healed out.

Summary of Results.

1. *Sheep* : —

   (*a*) Fifteen sheep were inoculated with this strain, and all developed typical bluetongue reactions.

   (*b*) Seven died.

2. *Calves.*—Three calves reacted to the virus after it had been passed through two generations of sheep. The lesions were again very similar in all cases and like those noted in other experiments.

3. *Immunity* : —

   (*a*) Two sheep which recovered from this cattle virus were inoculated with virulent bleutongue virus recovered from a sheep and were found to be immune.

   (*b*) Five bluetongue vaccine sheep were inoculated and four proved to be completely immune, whilst one showed a slight reaction.

(2) EXPERIMENTS WITH KROMDRAAI VIRUS.

Description of the Outbreak.

One animal became affected in this herd.

History.—The owner noticed a sudden reduction in the milk yield of the affected cow and at the same time observed a somewhat lame and stiff gait. On the next day marked salivation developed. Sores were discovered on the buccal mucosa. Foot and mouth disease was suspected and the case reported as such.

The Case.—A mature Friesland cow in poor condition.

The cow was first examined on 18th March, 1933. It was found standing with the back arched and head hanging down. It would not feed and rumination was in abeyance. There was fairly marked salivation and a profuse nasal discharge. This discharge was mucoid in nature. The muzzle was hot, dry, and very red. In places, especially towards the upper lip, the epidermis appeared necrosed. The temperature was elevated (104·5°, morning).

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Fairly extensive superficial necrotic lesions were present on the dental pad, borders of the lips, and on the ventral aspect of the tongue. These areas were covered with a yellowish material.

The animal was distinctly lame, and the limbs in the region of the fetlocks swollen. The skin, particularly on the plantar aspect of the digit, was red and a small amount of partly dried exudative material on the surface. The teats were all very red, hot, painful, and covered with a thin tough scab.

This cow was kept under very close observation. About four days later the mouth lesions showed distinct signs of healing, but now an extensive dermatitis developed. A distinct reddening of the unpigmented portions was first seen, and a serous material appeared on the surface. At this stage the skin was distinctly sensitive to the sun, and the cow was usually found sheltering in the shade. Subsequently hard crusts and scabs formed on practically the entire skin. The animal was now in a pitiful condition and was frequently struck with blow flies, especially round the mouth and in the flanks.

Hard and thick scabs formed on the teats. The scabs on the teats, muzzle, and the skin subsequently peeled off.

The owner was greatly concerned with the condition of his beast and very carefully nursed it. There is no doubt that if it had not been for this care, the animal would have died. She ultimately made a complete recovery.

A sample of blood was collected on 20th March and the following experiments undertaken : —

SICK COW
Kromdraai

Sheep 16 R (?)
Sheep 17 R
Sheep 18 R
Sheep 19 R

Sheep 37302 R
Sheep 37452 R

Sheep 37454 R
Sheep 37679 R

Blue-Tongue Vac.

Sheep 36519 Neg.
Sheep 36538 Neg.
Sheep 36580 Neg.
Sheep 36870 Neg.
Sheep 36962 Neg.

Sheep 37691 R
Sheep 37810 R
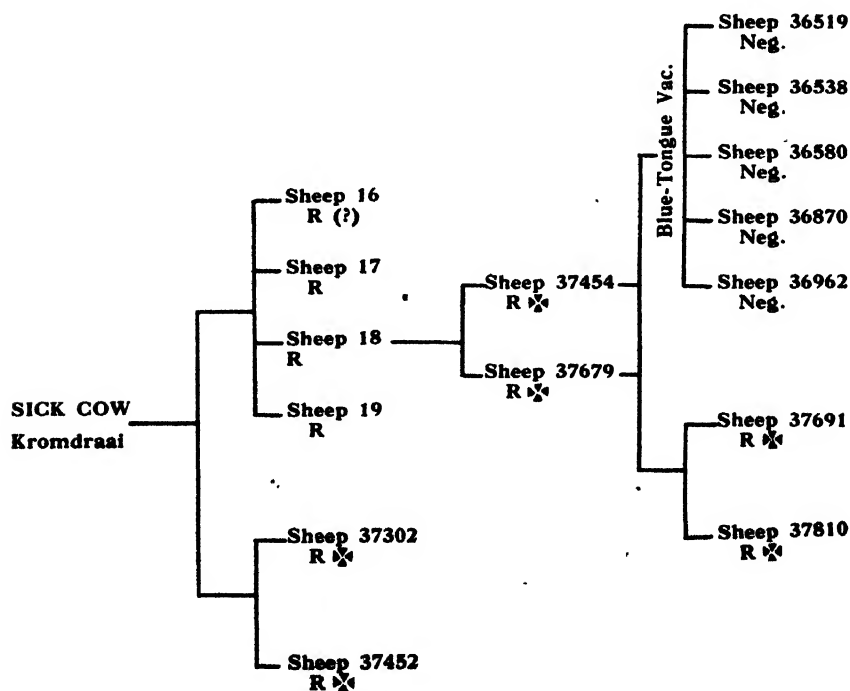
Table 2.

Experiments with Kromdraai Virus.

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep No. 16	22.3.33, 5 c.c. blood ex cow at Kromdraai subcutaneously. *Note.*—About equal quantity of glycerine added as preservative	Fairly mild and somewhat delayed reaction. I.P.— 9 days.
Sheep No. 17	„ „	Fairly mild acute reaction and recovery. I.P.—5 days.
Sheep No. 18	„ „	Fairly mild acute reaction and recovery. I.P.—6 days.
Sheep No. 19	„ „	Acute reaction and recovery. I.P.—5 days.
Sheep No. 37302	6.5.33, 2 c.c. blood ex cow Kromdraai, intravenously *Note.*—Equal quantity of glycerine added and stored at O.R. temperature for 47 days	Fatal subacute reaction. I.P.—9 days. Died on 22nd. *Note.*—In the later stages a marked discharge of ingesta through the nose (c.f. similar condition in horsesickness).
Sheep No. 37452	„ „	Fatal acute reaction. I.P.—9 days. Died on 14th day. *Note.*—A marked discharge of mucus through the nostrils in the later stages and a marked oedema of the glottis diagnosed at p.m. (*vide* appendix B).
Sheep No. 37454	6.5.33, 5 c.c. blood ex sheep No. 18 *Note.*—Blood collected on 6th day, preserved in O.G.C. and stored at O.R.T. for 39 days	Fatal peracute reaction. I.P.—4 days. Died on 9th day.
Sheep No. 37679	„ „	Subacute fatal bluetongue reaction. I.P.—7 days. Died on 17th day. *Note.*—On the 12th day a marked discharge of mucus from nostrils, subsequently complication of broncho-pneumonia developed, probably as a result of aspiration of food.
Sheep No. 37810	16.5.33, 1 c.c. fresh blood ex sheep No. 37454, subcutaneously *Note.*—Blood collected on 10th day	Fatal peracute reaction. I.P.—7 days. *In extremis* on 12th day and destroyed.
Sheep No. 37691	„ „	Fatal peracute reaction. I.P.—6 days. Died on 10th day. *Note.*—Temperature never very high.
Sheep No. 36519 (B. T.V. sheep)	„ „	No reaction.

Table 2—(contd.).

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep No. 36538 (B. T.V. sheep)	*16.5.33*, 1 c c. fresh blood ex sheep No. 37454, subcutaneously. *Note*—Blood collected on 10th day	No reaction.
Sheep No. 36580 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 36870 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 36862 (B. T.V. sheep)	,, ,,	,, ,,

Summary of Results.

In Sheep:—

(a) Ten susceptible sheep were inoculated with this cattle virus and all reacted.

(b) In the first generation two out of six sheep inoculated died. In subsequent generations four sheep were inoculated and all died.

(c) Five bluetongue vaccinated sheep were inoculated with this strain and all were found to be completely immune.

(3) EXPERIMENTS WITH DARLING CATTLE VIRUS.

Description of the Outbreak.

Only one animal, a black Friesland heifer, became affected in this outbreak. The heifer was first noticed to become lame; later salivation was observed. Sores were discovered in the mouth and the case reported as foot and mouth disease.

The Case.—The animal was examined on 12th April, 1933. Temperature: 106° (at 5 p.m.).

It was distinctly stiff and the lower portion of all four limbs somewhat swollen. A slight amount of salivation was observed. Superficial necrotic lesions were present on the dental pad, lips, and ventral surface of the tongue. The conical papillae were enlarged and their tips dirty grey in colour. Hyperaemia was masked by the natural pigmentation.

A sample of blood was collected and the following experiments undertaken:—

Table to indicate the transmission experiments with Darling cattle virus.

SICK HEIFER

Darling. Standerton

Sheep 37680
R

Sheep 37165
R

Sheep 37768
R

Sheep 37843
R

Blue-Tongue Vaccinated

Sheep 36622
Neg.

Sheep 36636
Neg.

Sheep 36822
Neg.

Sheep 36870
Neg.

Sheep 36904
Neg.

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Table 3.
Experiments with Darling Virus.

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep No. 37680	*6.5.33*, 5 c.c. blood ex cow Darling, intravenously *Note.*—Blood collected on 12.4.33, preserved in equal quantity of glycerine and stored at O.R. temperature	Subacute reaction and recovery. I.P.—4 days. *Immunity Test.*—On 30.5.33, i.e. 24th day, 1 c.c. virulent bluetongue blood ex sheet Novo—no reaction observed.
Sheep No. 37165	„ „	Fatal subacute reaction. I.P.—4 days. Died on 18th day.
Sheep No. 37768	*16.5.33*, 1 c.c. fresh blood ex sheep No. 37680, subcutaneously *Note.*—Blood collected on 10th day	Fatal acute reaction. I.P.—4 days. Died on 11th day. *Note.*— Extensive subcutaneous oedema and torticollis in later stages.
Sheep No. 37843	„ „	Acute reaction and recovery. I.P.—8 days. *Immunity Test.*—Tested as sheep No. 37680 and no reaction observed.
Sheep No. 36622 (B. T.V. sheep)	„ „	No reaction.
Sheep No. 36636 (B. T.V. sheep)	„ „	„ „
Sheep No. 36822 (B. T.V. sheep)	„ „	„ „
Sheep No. 36870 (B. T.V. sheep)	„ „	„ „
Sheep No. 36904 (B. T.V. sheep)	„ „	„ „

Summary of Results.

1. *In Sheep.*—Four normal sheep were inoculated with blood from this heifer and all developed typical bluetongue reactions. Two animals died.

2. *Immunity* : —
   (a) Two recovered sheep were immune to a virulent bluetongue virus recovered from a sheep.
   (b) Five bluetongue vaccine sheep which were inoculated with this cattle virus and were found to be completely resistant.

4. EXPERIMENTS WITH ELANDSLAAGTE CATTLE VIRUS.

Description of the Outbreak.

History.—Only one animal became affected in this herd. According to the owner, this heifer suddenly developed an illness on 23rd April. He observed a blood-stained mucous discharge from the nose. The lower portion of the head appeared swollen, and the hind limbs, from the hocks to the claws, were also swollen. On these parts superficial skin lesions were observed, from which a serous exudate escaped, and which later actually bled. The case was reported as an unknown disease, with strong suspicion of foot and mouth disease.

The Case.—The animal, an 18-months-old Friesland heifer, was examined on 25th April. It appeared slightly dull and did not feed. Rumination was in abeyance. The temperature was elevated (105·8°). Slight salivation was observed, and there was a marked nasal discharge. The discharge was slightly blood-stained and contained a few small pieces of coagulated blood.

Numerous dark red spots and areas (petechiae and ecchymosis) were seen on the injected mucosa of the nasal cavities, inner surfaces of the lips, cheeks, and tongue (particularly on the ventral surface of the apex). There was a slight watery discharge from both eyes, and numerous petechiae and ecchymoses were present in the mucosa.

The gum at the roots of the incisor teeth appeared excoriated and covered with dark grey coloured material. The underlying tissues bled easily. The hard palate appeared somewhat swollen, excoriations in the sulci, and the rugae markedly injected. The superficial necrotic process in the sulci was most extensive, and practically the entire palate was involved.

The vagina was markedly swollen and the mucosa reddened with numerous petechiae and ecchymoses. A fairly extensive, bleeding skin lesion was present on the side of the vagina. The hind limbs were swollen and a number of skin lesions similar to that near to the vagina were present.

Bloodsmears were examined, but no organisms were seen.

The heifer died that same night. Unfortunately, a post-mortem examination was impossible. A sample of blood was taken, and the following experiments undertaken:—

```
                                          ┌─ Sheep 37426
                                          │   R
                                          │
   Blood-sick heifer, ───────────────────┤
      Elandslaagte                        │
                                          │
                                          └─ Sheep 37335
                                              R
```
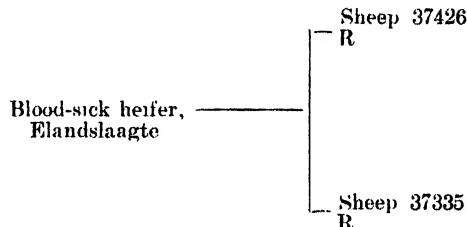
Table 4.

Experiments with Elandslaagte Virus.

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep No. 37335	*26.4.33*, 5 c.c. defibrinated blood ex heifer, Elandslaagte *Note.*—Blood collected on 25.4.33	Subacute reaction and recovery. I.P.—4 days. *Immunity Test.*—On 22.5.33, i.e. 27th day, 1 c.c. virulent bluetongue blood ex sheep Novo, subsubcutaneously. No reaction.
Sheep No. 37426	,, ,,	Fatal subacute reaction. I.P.—7 days. *In extremis* on 18th day and destroyed. In the later stages extensive subcutaneous oedema and marked oedema of the tongue which protruded prominently. Also discharge ingesta through nostrils.

Summary of Results.

1. *In Sheep.*—Two sheep were inoculated with blood from this case, and both developed severe bluetongue reactions. The one animal was destroyed *in extremis*.

2. *Immunity.*—The sheep which recovered from the above reaction proved to be immune to Novo sheep strain of virulent bluetongue.

(5) EXPERIMENTS WITH NOVO CATTLE VIRUS.

Description of the Outbreak.

History.—The disease made its appearance in a herd of Friesland milking cows on the above farm. In all five cases were observed. The owner suspected foot and mouth disease. The outbreak was first investigated by C. C. Wessels, Government Veterinary Officer. The usual description of the disease was given, viz., stiffness, followed by marked salivation, sores in the mouth, and later the teat and skin lesions.

Description of Cases.—The affected cows were again examined on 26.4.33, and most were now in a stage of recovery. The lesions noted in two of the cases deserve special mention. In one of these, the epidermis on a small unpigmented portion of the skin of the upper flank was peeling off (*vide* Fig. 12). The owner was very definite that this condition of the skin was associated with this stomatitic disease, evidence of which was still seen on the ventral surface of the tongue, where the mucosa along the frenum linguae was thickened and covered with a scab (*vide* Fig. 6). There appears to be little doubt that the dermatitis observed in this disease developed in this case, and the lesion was confined to a fairly small unpigmented portion of skin.

In the other case well-marked lesions were still present on the ventral surface of the tongue. The mucosa was thickened and covered with a fairly tough brown coloured deposit (*vide* Fig. 7). The epidermis of thinner portions of the skin, e.g. at the root of the tail, was peeling off (*vide* Fig. 8). Hard scabs were still adherent to the teats. A sample of blood was taken from this case, and the following transmission experiments were undertaken:—

Table to indicate the transmission experiments with Novo cattle virus.

SICK COW

Novo, Wepener

Sheep on the Farm

Sheep A
Reacted

Sheep B
R

Sheep 37428
R

Sheep 37303
R
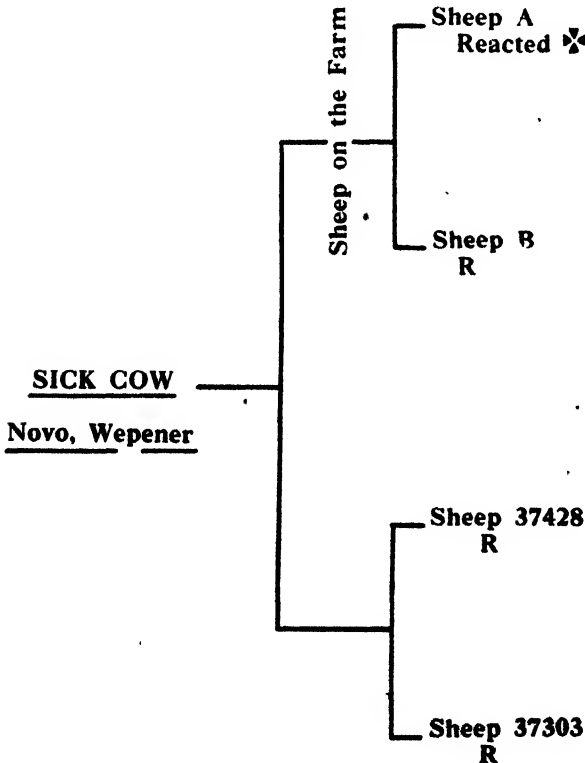
Table 5.

Experiments with Novo Cattle Virus.

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep A......	*26.4.33*, 5 c.c. fresh blood ex cow Novo	Fatal acute reaction. Died on 10th day. *Note.*—This animal was kindly provided by the owner and left on the farm. Mr. C. C. Wessels examined the animal after the onset of the reaction.
Sheep B.....	,, ,,	Acute reaction and recovery.
Sheep No. 37303	*27.4.33*, 10 c.c. defibrinated blood ex cow Novo, intravenously	Acute reaction and recovered. I.P.—10 days. *Note.*—Somewhat delayed reaction. *Immunity.*—On 22.5.33, i.e. 25th day, 1 c.c. virulent bluetongue blood ex sheep at Novo—no reaction observed.
Sheep No. 37428	,, ,,	Subacute reaction and recovered. I.P.—2 days. *Immunity.*—Tested as sheep No. 37303, and no reaction observed.

Summary of Results.

In Sheep.—Four sheep were inoculated with blood from one of the Novo cattle cases and all reacted. One died.

Immunity.—Two of the above sheep were inoculated with bluetongue virus recovered from a sheep on this farm and were found to be immune.

(6) EXPERIMENTS WITH MONTAGUE CATTLE VIRUS.

Description of the Outbreak.

Only one case was observed in this herd, and the owner suspected foot and mouth disease. The affected animal was a dry shorthorn cow. The typical stiffness was seen in the early stages, and subsequently mouth lesions and a swelling of the lower parts of the limbs were observed. The animal was examined on 26th of April, i.e. 12 days after the first symptoms had appeared. The mouth lesions were found to be practically healed, but evidence of the necrosis on the ventral surface of the tongue was still present. The mucosa along the frenum linguae appeared thickened, somewhat folded and covered with a brown coloured deposit. The epidermis of portions of the skin, e.g. at the root of the tail, was peeling off. No teat lesions were observed in this case. Blood was collected, and the following transmission experiments undertaken :—

The Transmission Experiments with Montague Cattle Virus.

Sheep 37174
R.

Sick cow,
Montague, Smithfield——

Sheep 37229
R.

471

<div align="center">

Table 6.

Experiments with Montague Virus.

</div>

No. of Animal.	Date of Inoculation and Source of Virus.	Result.
Sheep No. 37174	*28.4.33*, 10 c.c. blood ex cow, Montague *Note.*—Blood collected on 26.4.33 only defibrinated	Subacute reaction and recovery. I.P.—5 days. *Immunity Test.*—On 22.5.33, i.e. 25th day, 1 c.c. virulent bluetongue blood ex sheep Novo, subcutaneously. No reaction observed.
Sheep No. 37229	„ „	Subacute reaction and recovery. I.P.—5 days. *Immunity Test.*—Same as with sheep No. 37174. No reaction observed.

<div align="center">

Summary of Results.

</div>

Two sheep were inoculated with this strain, and both reacted and subsequently proved to be immune to bluetongue virus recovered from a sheep.

<div align="center">

(7) Experiments with Onlangs Cattle Virus.

History of the Outbreaks.

</div>

Three animals of a nondescript type were affected in this herd.

According to the owner the first case was observed on 4th of April. This animal suddenly became ill; she showed no inclination to feed and slight stiffness was observed. On the next day another case was discovered. By the 7th both animals salivated and sores were discovered in the mouths and teats. One of the affected cows. died on the 8th. The occurrence of this unknown disease caused anxiety in the neighbourhood and a virulent form of foot and mouth disease was suspected.

The herd was inspected on 10th April, 1933, and the following cases were found : —

Case No. 1.—A red and white shorthorn cow in poor condition, about 8 years old, in milk. The cow was found lying down. When forced to rise it only stood for a few minutes. The feet were apparently very painful, and the animal could not support itself when one foot was raised for examination.

The temperature was only slightly elevated (103·8° at 4 p.m.). Foaming at the mouth was observed. The muzzle was very red and partly covered with a brown scab which was firmly adherent to the underlying tissues. Similar lesions were present at the opening of the nostrils, which were also encrusted with partially dessicated mucus. The lips were swollen and along the borders covered with a yellowish deposit. The conical papillae, especially at the commissures of the mouth, appeared swollen and their tips were necrotic. Extensive necrotic lesions were present on the dental pad, in the groove between the upper lips and dental pad, and on the gums along the posterior aspect of the incissor teeth. The tongue was swollen and red, the mucosa of the ventral surface was excoriated. All the teats similarly excoriated and covered with a thin, tough scab. The lower part of all four feet from above the fetlock to the claws were swollen and the skin, especially on the plantar surface, red, hot, and painful.

Pathological-anatomical changes noted in this animal : —

    (1) Necrosis of the epithelium of the muzzle, external nares, dental pad, upper and lower lips, gums, ventral aspect of the tongue.

    (2) Diffuse superficial necrosis of the epidermis of teats.

    (3) Acute dermatitis (stadium rubosa) of the skin of the digits.

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</div>

Blood was taken from this animal, but as indicated on the following table the presence of bluetongue virus could not be established in the series of transmission experiments:—

Table to indicate transmission experiments undertaken with suspected virulent material, case 1, Onlangs.

SICK COW. Case 1.

Onlangs, Frankfort.

Blue-Tongue Vacc.

Calf 5206
Neg.

Calf 5263
Neg.

Sheep 35530
Neg.

Sheep 35515
Neg.

Sheep 35550
Neg.

Sheep 35778
Neg.

Sheep 37069
Neg.

Sheep 37081
Neg.

Sheep 37078
Neg.

Sheep 37070
Temperature

Sheep 37319
Neg.
(✕ Peritonitis)

Sheep 37430
Neg.
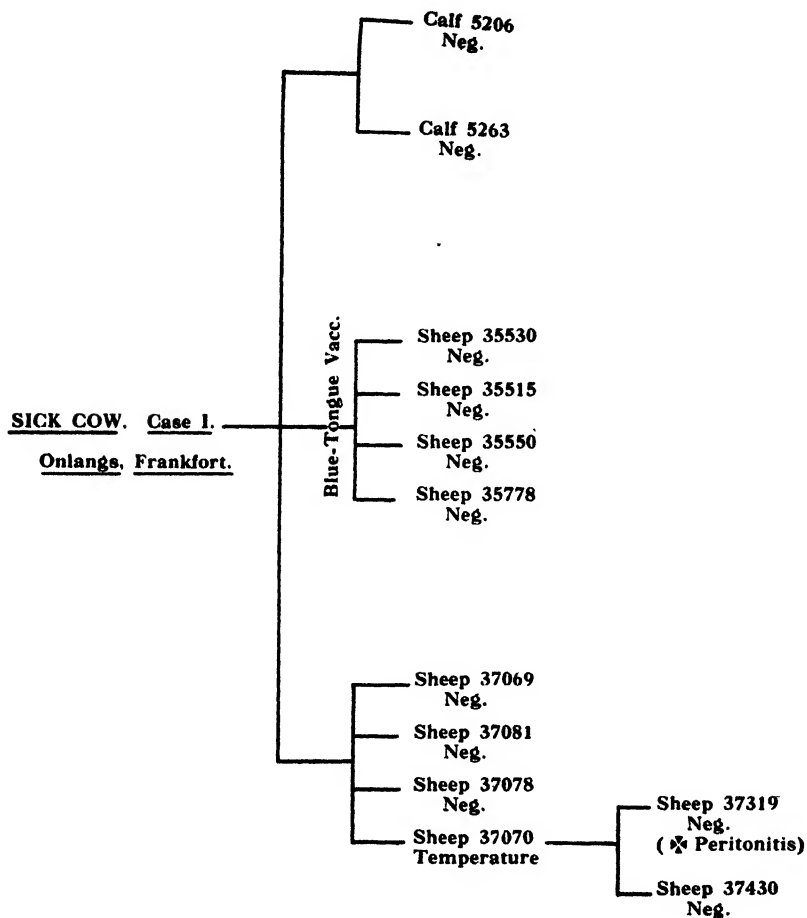
Table 7 (a).

Experiments with Onlangs Virus, case 1.

No. of Animal.	Reaction Tests.		Immunity Tests.	
	Date of Inoculation and Source of Virus.	Result.	Date of Test and Source of Virus.	Result.
Calf No. 5206	*19.4.33*, 5 c.c. blood ex case (i), Onlangs, subcut. *Note.*—blood in O. G.C. and stored for 14 days at O.R.T.	No reaction	*4.5.33*, 10 c.c. blood ex sheep No. 37265 subcut. (Welgezegend cattle virus)	Reaction. [Vide expt. 1 (c)].
Calf No. 5263	,,	,,	,,	,,
Sheep No. 35530 (B. T.V. sheep)	*19.4.33*, 1 c.c. blood of case (i), Onlangs, subcutaneously	,,	—	—
Sheep No. 35515 (B. T.V. sheep)	,,	,,	—	
Sheep No. 35550 (B. T.V. sheep)	,,	,,	—	—
Sheep No. 35778 (B. T.V. sheep)	,,	,,	—	—
Sheep No. 37069	,,	No reaction. (Vague temperature apparently of no significance)	*10.5.33*, 1 c.c. virulent B.T.V. blood ex sheep Novo	Subacute reaction. I.P.—4 days.
Sheep No. 37081	,,	No symptoms of bluetongue. (Rise in temperature and animal died on 4th day)	—	—
Sheep No. 37078	,,	No reaction. (Vague temperature, but no other symptoms)	*10.5.33*, as sheep No. 37069	Fatal acute reaction. I.P.—4 days. Died on 15th day.
Sheep No. 37070	,,	No reaction (?), slight rise in temperature on 6th and 7th days	,,	Acute reaction. I. P.—6 days.
Sheep No. 37319	*26.4.33*, 5 c.c. blood ex sheep No. 37070 subcut. *Note.*—blood collected on 7th day when a slight rise in temperature was noted	No symptoms of bluetongue. On 11th day rise in temperature and died same day. Cause of death perforation peritonitis (*vide* appendix C)	—	—
Sheep No. 37430	,,	No reaction	*22.5.33*, 1 c.c. virulent. B.T. blood ex sheep Novo	Subacute reaction. I.P.—6 days.

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Summary of Results.

It was impossible to demonstrate the presence of virus in blood taken from this case. That the sheep were susceptible is proved by the reactions brought about by Novo sheep virus.

In several animals vague rises in temperature was recorded, but apparently this was of no significance, since blood taken from one such case was tested on sheep and found to be avirulent.

Case No. 2.—This was a more recent case and this heifer was first noticed to be ill on 9th April. The lesions were mild when compared to those seen in Case No. 1. The necrotic lesions were confined to a fairly small area of the dental pad, and only a few papillae showed pathological changes. The temperature was somewhat elevated (104·6°). A sample of blood was collected on the 10th and the following experiments undertaken:—

Table to indicate the transmission experiments with cattle virus recovered from case 2, Onlangs.

Table 7 (b).

No. of Animal.	Reaction Tests.		Immunity Tests.	
	Date of Inoculation and Source of Virus.	Result.	Date of Test and Source of Virus.	Result.
Sheep No. 37678	*6.5.33*, 5 c.c. blood ex cattle, case (ii), Onlangs, intravenously *Note.*—Blood preserved in O.G.C. and stored for 27 days	Acute reaction. I.P.—3 days	*30.5.33*, 1 c.c. virulent bluetongue blood ex sheep Novo subcutaneously	Very doubtful reaction, i.e. slight rise in temperature and slight injection of buccal mucosa.
Sheep No. 37405	,,	Fairly mild acute reaction. I.P.—5 days	,,	No reaction.
Sheep No. 37730	*16.5.33*, 1 c.c. fresh blood ex sheep No. 37678 subcutaneously	Fairly mild acute reaction. I.P.—7 days	*9.6.33*, 1 c.c. virulent bluetongue blood ex sheep Novo, subcutaneously	,,
Sheep No. 37861	,,	Subacute reaction. I.P.—5 days	,,	,,
Sheep No. 36631 (B. T.V. sheep)	,,	No reaction	—	—
Sheep No. 36674 (B. T.V. sheep)	,,	,,	—	—
Sheep No. 36852 (B. T.V. sheep)	,,	,,	—	—
Sheep No. 36853 (B. T.V. sheep)	,,	,,	—	—
Sheep No. 36918 (B. T.V. sheep)	,,	,,	—	—

Summary of Results.

1. *In Sheep.*—Four susceptible sheep were inoculated with blood collected from this case (case 2) and all developed typical reactions.

2. *Immunity* :—

    (a) Five bluetongue vaccine sheep were immune to this cattle virus.

    (b) The four sheep which had recovered from the reactions produced by the cattle virus were tested with virulent bluetongue virus recovered from a sheep and three were immune. In one animal a very doubtful reaction resulted.

(8) EXPERIMENTS WITH SWARTLAND VIRUS.
Description of Cases.

On the 11th May, 1933, the herd of 275 mixed cattle on this farm was examined and 25 animals were found affected with a disease of which the following is a description: " The animal stands dull and listless with the head hanging and the ears drooping. The feet are somewhat bunched together. There is marked lachrymation and profuse salivation, the saliva hanging in strings from the lips. There is a discharge of thick mucus from the nose.

The breath is extremely foetid. The borders of the lips are excoriated and covered with a diphtheritic deposit. The frenum linguae is ulcerated and covered with a diptheritic deposit. In most cases a similar lesion extends along the lateral portion of the fixed part of the tongue; the lesion is most extensive. In one case the whole dorsum of the tongue was necrotic and peeled off when handled. The palate in some cases also showed similar lesions, which, however, were less extensive.

The foot lesion was very similar in each case. It begins as an acute coronitis especially towards the anterior part of the cleft. It then extends around the coronet and through the interdigital space, and appears to be particularly acute on the bulbs of the heel. No very recent lesions were found in the various cases. The skin above the coronet was found to be in a state of dry necrosis. The necrotic skin was firmly adherent to the underlying tissues from which it was difficult to remove, leaving a bleeding surface."

Blood was collected from two of these cases, and the following transmission experiments undertaken:—
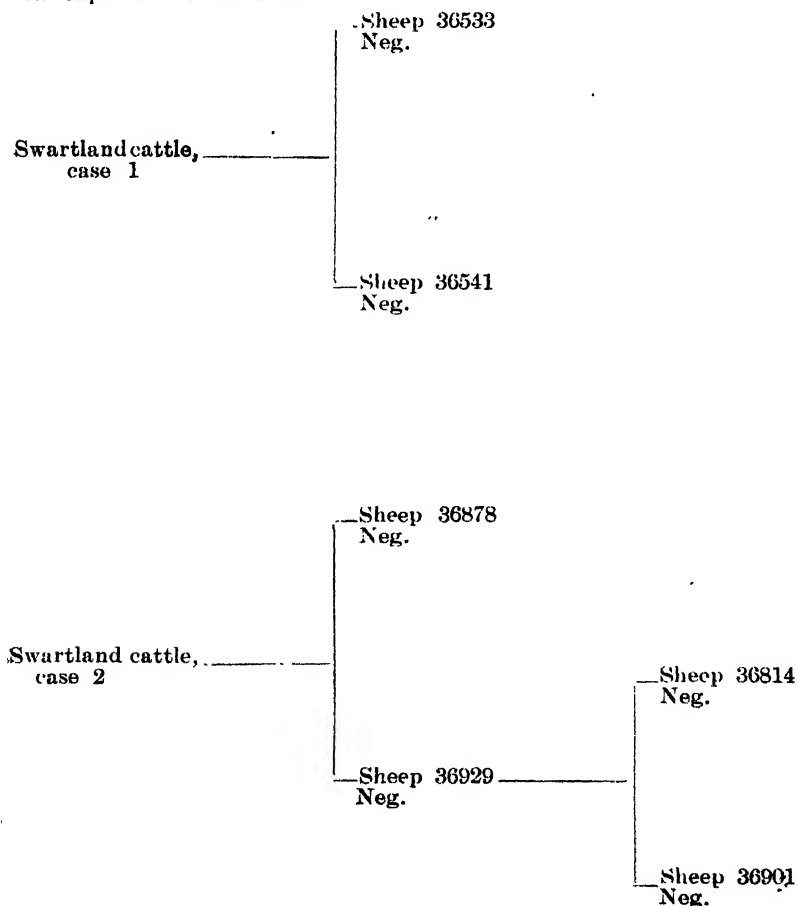
Table 8.

Experiments with Swartland Virus.

No. of Animal.	Date of Inoculation and Origin of Virus.	Result.	Immunity Test: Date and Origin of Virus.	Result.
Sheep No. 36533	*12.5.33*, 5 c.c. defibrinated blood ex case 1, Swartland	No reaction	*30.5.33*, 1 c.c. blood ex sheep No. 37331, i.e. Welgezegend cattle virus	Acute reaction and recovery. I.P.—6 days.
Sheep No. 36541	,,	,,	,,	,,
Sheep No. 36878	*27.5.33*, 5 c.c. defibrinated blood ex case 2, Swartland Note.—blood stored in ice chest for 15 days.	,,	*19.6.33*, 2 c.c. blood ex sheep No. 37328, i.e. Welgezegend cattle virus	Subacute reaction and recovery. I.P. —4 days.
Sheep No. 36929	,,	No reaction. (Slight rise in temp. on 12th day)	,,	Mild reaction. I.P. —4 days.
Sheep No. 36814	*9.6.33*, 2 c.c. blood ex sheep No. 36929	,,	*6.7.33*, 1 c.c. blood ex bluetongue sheep Novo	Subacute reaction and recovery. I.P. —6 days.
Sheep No. 36901	,,	,,	,,	Subacute reaction. I.P.—6 days.

Summary of Results.

It was impossible to demonstrate the presence of an infective agent in the blood of these cases. The avirulence of the blood can probably be attributed to the fact that both were old cases.

(9) EXPERIMENTS WITH A STRAIN OF BLUETONGUE VIRUS RECOVERED FROM A SHEEP IN A FIELD OUTBREAK ON THE FARM NOVO, WEPENER DISTRICT.

History.—Sheep first noted ill on 24.4.33.

Description.—Examined on 26.4.33. A marked muco-haemorrhagic discharge from the nose was present and fairly extensive excoriations were noted on the nose, lips, tongue, cheeks, and vulva. The buccal mucosa was markedly injected.

A large sample of blood was taken from this animal and preserved in O.G.C. mixture. This sample proved to be virulent, as will be seen in the series of experiments given in Table 9. Virus contained in this sample was used for testing the immunity of sheep which had recovered from the reactions produced by cattle strains.

NOTE.—It will be noticed that a large number of sheep was inoculated with this virus; many of these sheep were used as contacts and controls of batches of sheep used in the experiments with the cattle strains. Some of these showed vague temperature reactions, and it became necessary to test their immunity.

Table to indicate the transmissions undertaken with bluetongue virus ex a natural case in a sheep at Novo.

```
                                                    Sheep 37068
                                                        R
                                                    Sheep 37069
                                                        R
                                                    Sheep 37070
                                                        R
                                                    Sheep 37073
                                                        R
                                                    Sheep 37076
                                                        R
                                                    Sheep 37079
                                                        R �҈
                                                    Sheep 37078
                                                        R �҈
                                                    Sheep 37080
                                                        R
                                                    Sheep 37083
                                                        R
                                                    Sheep 37084
                                                        R
                                                    Sheep 37086
                                                        R �҈
                                                    Sheep 37088
                                                        R
Natural Case of                                     Sheep 37089
Blue-Tongue (Sheep)                                     R ✓
Novo, Wepener                                       Sheep 37090
                                                        R
                                                    Sheep 37168
                                                        R ✓
                                                    Sheep 37200
                                                        R
                                                    Sheep 37814
                                                        R
                                                    Sheep 36901
                                                        R

                                                    Sheep 35520
                                                      Slight R
                       Blue-Tongue Vaccinated       Sheep 35533
                                                        Neg.
                                                    Sheep 35793
                                                       Neg.(?)
                                                    Sheep 35799
                                                        Neg.
                                                    Sheep 35801
                                                        Neg.
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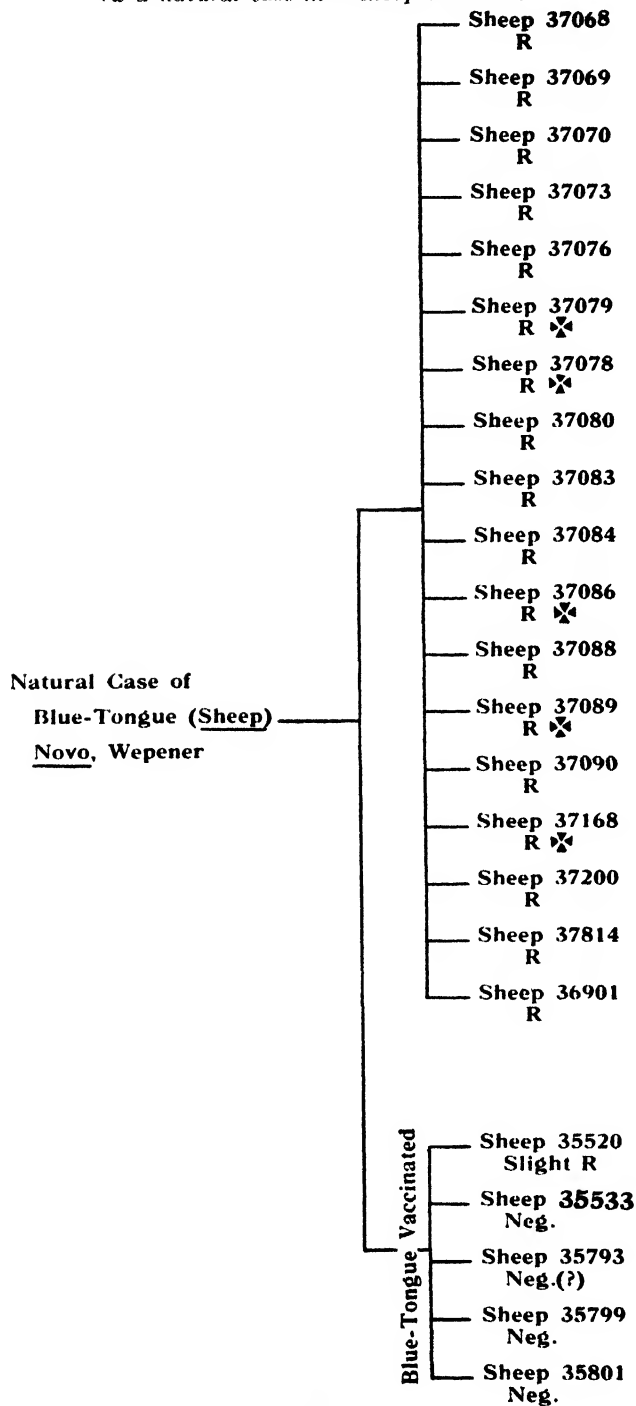
Table 9.

Experiments with Virulent Bluetongue Virus, ex Sheep, in a Field Outbreak at Novo, Wepener, O.F.S.

No. of Animal.	Date of Inoculation and Source of Virus.	Results.
Sheep No. 37168	*28.4.33,* 10 c.c. defibrinated blood ex sheep at Novo, intravenously *Note.*—Blood collected on 26.4.33	Fatal acute reaction. I.P.—3 days. Died on 11th day.
Sheep No. 37200	„ „	Subacute reaction and recovery. I.P.—6 days. *Immunity Test.*—On 25th day 1 c.c. fresh blood ex sheep No. 37328 (Welgezegend cattle virus). No reaction observed.
Sheep No. 37068	*10.5.33,* 1 c.c. blood ex sheep Novo, subcutaneously *Note.*—Blood collected on 26.4.33 and preserved in O.G.C. and stored in refrigerator	Subacute reaction and recovery. I.P.—4 days.
Sheep No. 37069	„ „	Subacute reaction and recovery. I.P.—2 days.
Sheep No. 37073	„ „	Mild peracute reaction and recovery. I.P.—4 days. *Note.*—Only temperature and injection of mucosa of buccal cavity.
Sheep No. 37070	„ „	Subacute reaction and recovery. I.P.—4 days.
Sheep No. 37076	„ „	Acute reaction and recovery. I.P.—5 days.
Sheep No. 37078	„ „	Acute reaction: Complication of peritonitis, the result of thermometer perforation of rectum. I.P.—5 days. Died on 12th day, result of peritonitis complication. *Note.*—Extensive oedema of subcutis noted on 8th day.
Sheep No. 37079	„ „	Fatal acute reaction. I.P.—5 days. Died on 14th day.
Sheep No. 37080	„ „	Acute reaction and recovery. I.P.—5 days.
Sheep No. 37083	„ „	Subacute reaction and recovery. I.P.—5 days.
Sheep No. 37084	„ „	„ „ „
Sheep No. 37086	„ „	Peracute fatal reaction. I.P.—5 days. Died on 11th day.

Table 9—(contd.).

No. of Animals.	Date of Inoculation and Source of Virus.	Results.
Sheep No. 37088	*10.5.33*, 1 c.c. blood ex sheep Novo, subcutaneously *Note.*—Blood collected on 26.4.33 and preserved in O.G.C. and stored in refrigerator	Subacute reaction and recovery. I.P.—5 days.
Sheep No. 37089	,, ,,	Fatal acute reaction. I.P.—8 days. Died on 14th day.
Sheep No. 37090	,, ,,	Acute reaction and recovery. I.P.—7 days.
Sheep No. 35520 (B. T.V. sheep)	*22.5.33*, 1 c.c. blood ex sheep Novo	Mild acute reaction and recovery. I.P.—5 days Slight injection of mucus membrane of mouth noted on 7th day.
Sheep No. 35533 (B. T.V. sheep)	,, ,,	No Reaction.
Sheep No. 35801 (B. T.V. sheep)	,, ,,	Abortive reaction, i.e. only a rise in temperature. I.P.—5 days.
Sheep No. 35793 (B. T.V. sheep)	,, ,,	No reaction.
Sheep No. 35799 (B. T.V. sheep)	,, ,,	,, ,,
Sheep No. 36901	*6.6.33*, 1 c.c. blood ex sheep Novo	Subacute reaction and recovery. I.P.—6 days. *Immunity.*—(On 22.7.33 2 c.c. blood ex sheep No. 36812, i.e. Kromdraai cattle virus), subcutaneously and no reactions.
Sheep No. 36814	,, ,,	Subacute reaction. I.P.—6 days. *Immunity.*—Same as sheep No. 37901 and no reaction.

Summary of Results.

(1) *In Sheep.*—Eighteen normal sheep were inoculated with this strain of blue-tongue virus recovered from a sheep. All reacted and three died. The reactions were typical and in all respects resembled those which resulted from the various cattle strains.

(2) *Immunity*:—

(a) Three of the sheep which recovered from the reactions produced by this sheep strain received cattle virus (Welgezegend and Kromdraai strains) and developed no reactions.

(b) Five bluetongue vaccine sheep were tested with this virus. Three proved to be completely immune. In one a very mild reaction developed, i.e. slight injection of buccal mucosa and a rise in temperature. In the remaining sheep only a rise in temperature was observed [abortive reaction(?)].

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MISCELLANEOUS EXPERIMENTS.
Experiments 10.
(a) The Presence of Bluetongue Virus in Foetal Material.

Two ewes (Nos. 37691 and 37810) used in experiments 3 (Kromdraai cattle virus) were found to be pregnant at post-mortem examination. Material was collected aseptically from these foetuses and the following experiments undertaken :—

```
                                          ┌─Sheep 36812
                                          │ R  �
Foetus 1 (from sheep 37691, i.e. ─────────┤
        Kromdraai cattle virus)           │
                                          └─Sheep 36984
                                            R
```

```
                                          ┌─Sheep 36779
                                          │ Neg.
Foetus 2 (from Sheep 37810, i.e. ─────────┤
        Kromdraai cattle virus)           │
                                          └─Sheep 36991
                                            Neg.
```

Table 10 (a).

To Indicate whether Foetal Material of Ewes Reacting to Bluetongue contain Virus.

No. of Animal.	Date of Inoculation and Source of Material.	Result.	Immunity Determination.	Result.
Sheep No. 36812	27.5.33, 1 c.c. fresh foetal blood ex sheep No. 37691, subcut.	Fatal acute reaction. Died on 13th day	—	—
Sheep No. 36984	27.5.33, 1 c.c. fresh foetal blood ex sheep No. 37691, subcut.	No reaction		
	17.6.33, 5 c.c. fresh foetal blood, same as used on 27.5.33, subcut. *Note.*—Blood stored in ice chest	Subacute reaction and recovery	12.7.33, 5 c.c. blood ex sheep No. 36812, subcut.	No reaction.
Sheep No. 36779	27.5.33, 5 c.c. saline emulsion of heart and spleen ex foetus sheep No. 37810, subcut.	No reaction		
	17.6.33, 5 c.c. saline of liver of above foetus, subcut.	No reaction	6.7.33, 5 c.c. blood ex sheep No. 36812 subcut.	Acute reaction. (Destroyed on 6th day for collection of path. material.

Table 10 (*a*)—contd.

No. of Animal.	Date of Inoculation and Source of Material.	Result.	Immunity Determination.	Result.
Sheep No. 36991	*27.5.33,* 5 c.c. saline emulsion of heart and spleen ex foetus sheep No. 37810, subcut.	No reaction.		
	17.6.33, 5 c.c. saline emulsion of liver of above foetus, subcut.	No reaction	6.7.33, 5 c.c. blood ex sheep No. 36812, subcut.	Acute reaction with recovery.

Summary of Results.

The blood of foetus 1 was active and contained virus. In this case 1 c.c. set up a reaction in one of two sheep. One animal did not react, but subsequently when 5 c.c. of the same foetal blood was inoculated a typical reaction followed. Later this sheep was found to be immune to the virus contained in the blood of the mother of the foetus. Foetus 2 was apparently sterile. The two sheep which were used for testing material from this foetus subsequently reacted to virus contained in the blood of one of the dams.

(b) *The duration of the Infection in the Blood of Sheep Recovering from a Bluetongue Reaction.*

Two sheep, Nos. 37464 and 37274, used in experiments 1 (c) were used for determining the above. Both of these animals developed typical bluetongue reactions. At 14-day intervals blood was collected from these animals and inoculated into normal sheep. The following is a summary of the results:—
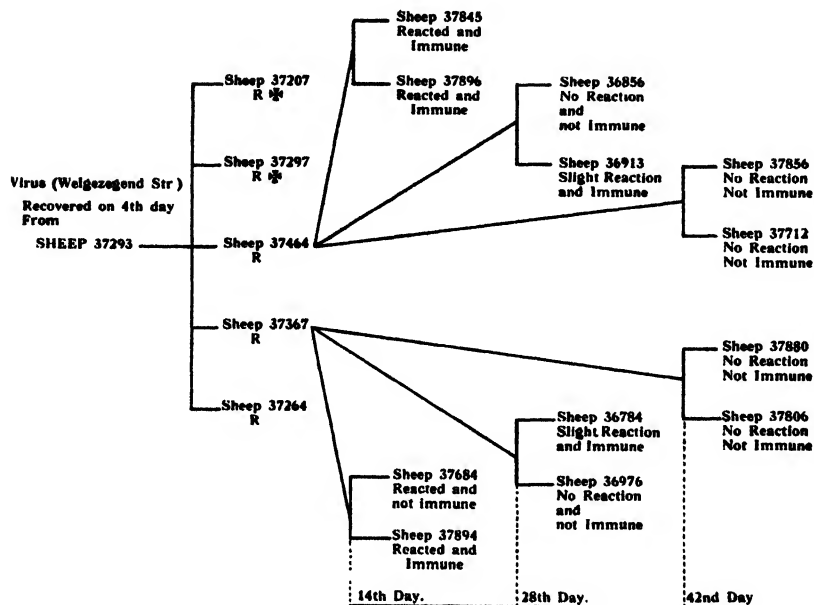
Table 10 (b).

To Determine the Duration of Infection in the Blood of Sheep Recovering from a Bluetongue Reaction.

No. of Animal.	Date of Inoculation and Source of Virus.	Result.	Date of Immunity Test and Source of Virus.	Result.
Sheep No. 37845	16.5.33, 5 c.c. blood ex sheep No. 37464 (14 days after infection)	Mild acute reaction and recovery	9.6.33, 1 c.c. Novo strain of B.T. virus ex sheep	No reaction.
Sheep No. 37896	,,	,,	,,	,,
Sheep No. 37684	16.5.33, 5 c.c. fresh blood ex sheep No. 37367 (14 days after infection)	Subacute reaction and recovery	,,	Mild acute reaction (i.e. rise in temperature and hyperaemia mucosa of buccal cavity.
Sheep No. 37894	,,	,,	,,	No reaction.
Sheep No. 36856	30.5.33, 5 c.c. fresh blood ex sheep No. 37464 (28 days after infection)	No reaction	19.6.33, 2 c.c. blood ex sheep No. 37464 *Note.*—Blood collected on 7th day	Acute reaction and recovery.
Sheep No. 36913	,,	Somewhat delayed mild acute reaction	,,	No reaction.
Sheep No. 36784	30.5.33, 5 c.c. fresh blood ex sheep No. 37367 (28 days after infection	Mild acute reaction	19.6.33, 2 c.c. blood ex sheep No. 37367 *Note.*—Blood collected 7th day after infection	,,
Sheep No. 36976	,,	No reaction	,,	Fatal acute reaction. Killed *in extremis* on 15th day.
Sheep No. 37856	13.6.33, 5 c.c. blood ex sheep No. 37464 (42 days after infection)	,,	28.6.33, 10 c.c. Seitz filtrate = 2 c.c. whole blood ex sheep No. 36812. *Note.*— Kromdraai cattle strain.	No definite clinical reaction. Animal died on 9.7.32 and bluetongue was not diagnosed at p.m.
Sheep No. 37712	,,	,,	28.6.33, 2 c.c. blood ex sheep No. 36812	Slight acute reaction. *Note.*—afebrile but definite hyperaemia and excoriations.
—	—	—	18.7.33, 5 c.c. blood ex sheep No. 36812	No reaction.

Table 10 (*b*)—(contd.).

No. of Animal.	Date of Inoculation and Source of Virus.	Result.	Date of Immunity Test and Source of Virus.	Result.
Sheep No. 37880	*13.6.33*, 5 c.c. blood ex sheep No. 37367	No reaction	*28.6.33*, 2 c.c. blood ex sheep No. 36812	Mild acute reaction.
			18.7.33, 5 c.c. blood ex sheep No. 36812	No reaction.
Sheep No. 37806	*13.6.33*, 5 c.c. blood ex sheep No. 37367.	No reaction.	*28.6.33*, 10 c.c. Seitz filtrate : 2 c.c. blood ex sheep No. 36812	No reaction.
			18.7.33, 5 c.c. whole blood ex sheep No. 36812	Fatal acute reaction. Killed *in extremis* on 12th day.

Summary of Results.

It will be noticed that—

(1) the virus recovered on the fourth day after infection from sheep 37293 (Experiment 1) set up severe reactions in five sheep, and two died ;

(2) the blood of two of the recovered animals was still infective and active on the fourteenth day after infection. In each case reactions resulted in two sheep. Three of these animals were immune to the strain of Novo sheep virus, but apparently the immunity in one (sheep 37684) was weak, since this animal developed a mild reaction to this sheep strain of bluetongue virus;

(3) on the twenty-eighth day the blood from the same two animals in the same amounts was apparently less active since one of two inoculated sheep developed only slight reactions. These two animals proved to be immune to virus contained in a sample of blood taken on the seventh day from the original donors. The two non-reactors developed severe reactions to this sample of virus, and one died ;

(4) Blood collected on the forty-second day failed to set up perceptible reactions. These sheep were subsequently used for testing the activity of Seitz filtered blood. No definite reactions were observed in sheep 37856 and 37806, i.e. those which received filtered material. Sheep 37856, however, died, but the cause of death was obscure. Sheep 37806 was again tested with the same sample of whole blood, and a fatal acute reaction followed. Sheep 37712 and 37880, where the immunity was in the first instance tested with unfiltered blood from the same source, developed mild reactions, and subsequently were immune to a larger .dose of the same sample of virus material.

The above results indicate that—

(*a*) the blood of two of the sheep which were recovering from reactions produced by the Welgezegend cattle strain of virus was still slightly infective 28 days after the date of infection, but was definitely inactive in subcutaneous doses of 5 c.c. on the forty-second day ;

(*b*) as far as the pathogenicity is concerned, the virus present in the blood of these recovering animals became progressively less active.

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(c) *Experimental Infection of Animals by means of Intranasal Injection with Virus-containing Material.*

When experiments were commenced at Welgezegend, five calves were injected intranasally with material consisting of a mixture of blood, urine, milk and emulsified necrotic tissue from three affected cows. This experiment was undertaken with the express purpose of excluding foot and mouth disease. Four out of these five calves developed definite reactions. The infectivity with bluetongue virus in the blood of one of these calves was established in the subsequent experiments [*vide* Experiments 1 (b)].

In Experiment 1 (a) an attempt was made to confirm this method of intranasal infection in sheep. Two animals were accordingly injected intranasally with 5 c.c. blood from a reacting sheep. No reactions, however, became perceptible. This experiment was repeated at Onderstepoort, with the following results : —

Table 10 (c).

To Determine whether it is possible to Infect Sheep with an Intranasal Injection of Virus-containing Material.

No. of Animal.	Date of Infection and Nature of Infective Material.	Result.	Date of Immunity Test and Source of Material.	Result.
Sheep No. 37801	*7.7.33*, intranasal injection of a mixture 25 c.c. blood, bile and emulsified necrotic buccal mucosal scrapings ex sheep No. 33614, i.e. Kromdraai virus	Peracute fatal reaction. I.P.—3 days. Died on 10th day	—	—
Sheep No. 37879	,,	No reaction	*18.7.33*, 2 c.c. saline emulsion of spleen pulp ex sheep No. 37801	Acute reaction.
Sheep No. 37762	*7.7.33*, intranasal injection of 20 c.c. blood ex sheep No. 33614	,,	,,	Fatal peracute reaction. I.P.—2 days. Died on 8th day.
Sheep No. 37760	,,	,,	,,	Fatal acute reaction. I.P.—4 days. In moribund condition on 11th day and destroyed.

Summary of Results.

One sheep (No. 37801) became infected and reacted after an intranasal injction of virus-containing material.

(d) *Intramucosal Inoculation of Virus-containing Material.*

The pathological-anatomical changes seen in bluetongue clearly indicates an epitheliotropic nature of the virus. Distinct changes (*vide* pathology) become manifest in the mucous membranes.

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It was considered not unlikely that reactions on the buccal mucosa would be provoked at the site of inoculation of virus-containing material. The following experiments were therefore arranged:—

Sheep 23812 R.
This animal was inoculated with virus, *ex* Foetus 1, of Sheep 37691 [*vide* Experiment 10 (*a*)].

- -Calf 5201 React.
-Calf 5257 React.

— Sheep 36935 R.
—Sheep 37887 R ✠
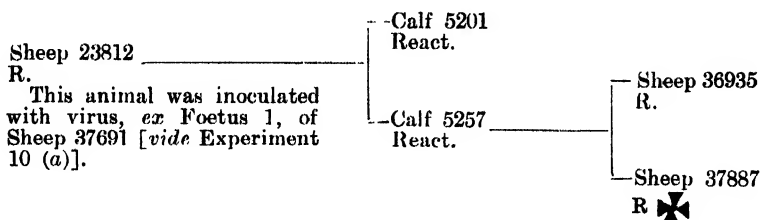
Table 10 (*d*).

To Determine the Result of an Intramucosal Inoculation of Virus Material.

No. of Animal.	Date, Method of inoculation, and Source of Material.	Result.
Calf No. 5201	*8.6.33*, Kromdraai cattle virus ex sheep No. 36812 as follows: (1) Left side of tongue: 0·5 c.c. buccal epithelium emulsion in saline, intramucosally; (2) Right side of tongue: 0·5 c.c. blood	Distinct rise of temperature after an incubation period of 3 days (*vide* Chart X). On the second day slight injection of the mucosa at the sites of inoculation was noticeable, but no lesions developed. On the 15th day small necrotic lesions were seen on the dental pad and inner surface of lower lip.
Calf No. 5257	„ „	Distinct rise in temperature on 4th day (*vide* Fig. XI). On the second day after inoculation slight reddening at the sites of inoculations on the tongue. Excoriations appeared on the mucosa of the upper and lower lips and at the angles of the mouth. These lesions appeared on the 6th day and persisted for 12 days. In the later stages they became somewhat ulcerative.
Sheep No. 36935	*13.6.33*, 5 c.c. blood ex calf No. 5257, subcutaneously	Subacute reaction and recovery. *Immunity Test.*—On 6.7.33 1 c.c. blood ex blue-tongue sheep Novo. Only a slight rise in temperature on 7th day was observed and this was apparently of no significance.
Sheep No. 37887	„ „	Fatal peracute reaction. Died on 7th day.

Summary of Results.

Contrary to expectations, no distinct local lesions developed at the site of inoculation on the tongues of the calves. However, general reactions followed, and this was similar as observed in calves in other experiments, where virus-containing material was injected intravenously, subcutaneously or intranasally.

The blood of one of the calves (5257) was infective as is illustrated by the reactions which developed in the sheep.

APPENDIX B.

Under this will be included all data of post-mortems and subsequent microscopical examinations of the various tissues collected. In each case reference will be made to the experimental number, about which full particulars will be obtained in Appendix A, the number of the sheep, date of death, number of days after injection of infected material which elapsed before death occurred, the etiological diagnosis with the significance of most important changes, followed by a summary of macroscopical and microscopical findings.

The post-mortems under review will be considered under two headings: (1) natural cattle cases, (2) experimental sheep cases.

1. *Natural Cattle Cases.*

These mainly refer to three Friesland bovines concerned in the outbreak at Welgezegend; these were destroyed for post-mortem examination.

(*a*) Black and white cow, condition rather poor, staring coat, killed for post-mortem.

Slight general anaemia and cachexia; swelling of the tongue fairly extensive, causing partial protrusion from the mouth; on dorso-lateral aspect of the tongue there is localised necrosis about 1¼ c.m. in diameter and about ¼ c.m. in depth, surrounded by a reddish zone; on the upper lip there are two superficial ulcers on the mucous membrane. The other organs showed no specific changes.

(*b*) Black and white cow, condition poor, coat staring, killed *in extremis*.

General anaemia and cachexia; fairly extensive deep-seated foetid partly gangrenous necrosis of the tongue, involving practically the whole of the left dorso-lateral aspect of the middle third—it is about 3 c.m. in depth in places, extending well into the muscular substance of the tongue; superficial ulcers on the mucous membranes of the lips and the dental pads; the superficial layers of the skin in the interdigital space show a shreaded appearance due to partial desquamation, leaving a reddish moist surface after removal; multiple circumscribed necrotic nodules in the lungs, varying in size from ½-1 c.m., with no evidence of encapsulation; localised sero-fibrinous pleuritis; linear fairly deep-seated ulcers on the pylorus, about 3 c.m. × ¼ c.m. × ¼ c.m., edges clean and sharply defined with a dark, reddish base and periphery; hyperaemia and a slight superficial dermatitis of the skin of the teats extending on to the udder.

Microscopical Examination (Specimen No. 13637).

Tongue.—In places some of the epithelial cells show "ballooning" and a loose infiltration with neutrophiles in others. The localised necrosis involves the whole of the epithelial layers, which has become disorganised and in many respects lost. The whole has become infiltrated with neutrophiles, which show necrobiosis. The cells underlying the necrotic material still show evidence of extensive cellular infiltration extending into the musculature of the tongue. The process is of the nature of an acute localised necrotic stomatitis characterised by the absence of any extensive proliferation of connective tissue.

Lip.—Similar lesions of necrosis and ulceration with infiltration of the deeper layers of the mucous membrane.

Pyloris.—Ditto.

Lung.—Shows multiple, fairly extensive localised necrotic areas without encapsulation at the periphery of these lesions, numerous bacteria in the form of filaments can be identified forming an extensive network, probably *B. necrophorus.*

(*c*) Black and white Fries cow, recovering from pseudo foot and mouth disease, killed for post-mortem.

Slight general anaemia and cachexia, superficial necrosis of the dental pad, several ulcers with hyperaemia of the periphery of the pylorus of the stomach, ulceration of the dorsum of the middle third of the tongue about 1 c.m. in diameter in the process of healing, characterised by sharply defined edges as if a circumscribed portion of the mucous membrane had been scooped out. No further pathological changes of a specific character was seen in this case.

No. of Expt.	No. of Sheep.	Death. Date.	Death. No. of Days after Injection.	No. of Days of Reaction.	Specimen No.	Etiology.	Macroscopical.	Microscopical.
1 (a)	37426	9.5.33	13	5	—	Killed in extremis, bluetongue (characterised by necrotic lesions in mucous membranes in stage of healing)	Excoriations nostrils; superficial ulcers, lower lip; cornitis; haemorrhagic cystitis; sub-endocardial haemorrhages; healed-out ulcer tip of tongue; hyperaemia oesophageal groove and petechiae along fold; oesophagostomum nodules, oedema peri-tracheal tissues and inter-mandibular space	No material.
1 (a)	37395	9.5.33	14	7	—	Bluetongue (characterised by multiple haemorrhages)	Slight oedema lungs; aspiration of food; acute fibrinous peritonitis secnel perforation of rectum; slight tumorsplenis; hyperaemia abomasum and intestine, hydro-thorax; sub-pleural ecchymoses; acute coronitis; perforation rectum	?
1 (a)	37077	1.5.33	12	10	13785	Bluetongue (characterised by multiple necrotic areas and hyperaemia of mucous membranes)	Small necrotic areas lips; localised hyperaemia lips; hyperaemia of papillae anterior portion tongue; hyperaemia pharynx and larynx, tumor splenis; acute haemorrhagic gastro-enteritis	?
1 (?)	37411	9.5.33	12	19	13810	Killed in extremis, bluetongue (characterised by haemorrhages and necrosis in stage of healing)	Oedema peri-tracheal tissues and intermandibular space; sub-epicardial haemorrhages; sub-mucous haemorrhages tongue; coronitis; excoriations skin; hyperaemia oesophageal grooves; petechiae small intestine and abomasum	?
1 (a)	37331	1.5.33	6	4	—	Bluetongue (characterised by necrosis in m m of mouth and nose, haemorrhages heart, and oedema lungs)	Petechiae epicardium and endocardium; hyperaemia and oedema lungs, petechiae and ecchymoses ucosa of upper and lower lips, and upper and lower gums and tongue; acute catarrhal enteritis	?

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2. Experimental Sheep Cases—(contd.).

No. of Expt.	No. of Sheep.	Death.		No. of Days of Reaction.	Specimen No.	Etiology.	Macroscopical.	Microscopical.
		Date.	No. of Days after Injection.					
1 (a)	37421	12.5.33	15	12	—	Bluetongue (characterised by necrosis in mouth and nose, haemorrhages heart, and oedema lungs)	Post-mortem changes fairly advanced; necrosis external nares; cyanosis mucosa mouth and skin; necrosis ventral aspect of tongue and along borders of tip; subendocardial, epicardial, and myocardial extravasations; ecchymoses mucosa conjunctiva; fairly marked oedema lungs; localised necrosis of hard palate; petechiae and ecchymoses mucosa bladder	No material.
1 (b)	37265	4.5.33	9	4	13795	Bluetongue (characterised by multiple petechiae mucous membranes and oedema subcutis)	Swelling and cyanosis at margins of lips, tongue and around hoofs; petechiae and hyperaemia small intestine and abomasum; hyperaemia large intestine; petechiae mucosa bladder; ecchymoses and extravasations epicardium; few ecchymoses both endocardium; slight hydropericardium; extensive oedema subcutis of intermandibular space and ventral aspect cervical region; haemorrhages in myocardium; fairly well marked hyperaemia liver, kidneys; slight hyperaemia and oedema lungs	*Lip* (skin): In places slight "ballooning" of epithelial cells; in the other places lesion more advanced, i.e. infiltration with neutrophiles, necrobiosis, and ulceration with infiltration of neutrophiles into the corium in places haemorrhages alone appears, also seen in the hair papilla around the root. *Lip* (papilla of mucous membrane): Haemorrhages in the corium and here and there deeper layers of the stratified epithelium show irregular vacuolation; at the apex some of the papillae show space filled with a granular or stringy material; badly stained with eosin. *Myocardium*: Well marked haemorrhages in epicardium and in the substance of the myocardium. *Lung*: Sut pleural and interstitial haemorrhages, and oedena and hyperaemia. No specific changes in liver, kidneys, and spleen.
1 (b)	37298	15.4.33	20	15	—	Killed *in extremis*, bluetongue (characterised by oedema of subcutis)	Slight general anaemia: oedema subcutis of intermandibular space, cervical region, and surrounding tissue of trachea; degeneration of liver and kidneys; ascites, few parasitic nodules	No material.

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						Diagnosis	Macroscopic	Microscopic
1 (e)	37328	11.5.33	16	13	13961	Bluetongue and sequelae of verminosis (characterised by necrotic lesions in mouth and coronitis)	General anaemia; localised necrosis skin (pressure of mosquito cage); localised hyperaemia gums and external nares; hyperaemia of coronets; marked haemonchosis; numerous parasitic nodules in intestines; impaction forestomachs; constipation; slight tumor splenis	No specific changes in spleen, liver, and myocardium.
1 (e)	37298	2.5.33	7	5	13790	Killed in *extremis*, bluetongue (characterised by haemorrhages and hyperaemia in mucous membranes)	Cyanosis lips and tongue; multiple petechiae and ecchymoses epicard, endocard, lips, tongue, small intestine and trachea, slight extravasations serosa of rumen; hyperaemia kidneys; slight hyperaemia myocardium; slight diffuse hyperaemia nasal mucosa; marked swelling lips and tongue; very slight oedema lungs	*Tongue*: Hyperaemia and haemorrhages in corium extending into the deeper layers; a number of the papillae of corium infiltrated with neutrophiles; epithelial layers in places show "ballooning"; some of the epithelial layers show slight changes in the staining intensity, infiltration with neutrophiles, and loss of the superficial epithelial cells in places. *Lips*: Few haemorrhages into the papillae with slight infiltration with neutrophiles. *Myocardium*: Small irregular haemorrhages. No specific changes in lymph glands, spleen, brain, thyroid, and kidneys.
1 (e)	37287	22.5.33	12	7	13905	Bluetongue (characterised by oedema and necrosis buccal cavity)	Slight hyperaemia coronets; extensive oedema subcutis; ascites; oedema tongue; degeneration liver; localised necrotic areas lateral, ventral, and dorsal aspects of tongue (not extensive); oedema lungs; degeneration myocardium	*Liver*: Few haemorrhages in places associated with infiltration with neutrophiles and in places with necrobiosis; also slight hyperaemia. No specific changes in *myocardium* and *kidneys*.
1 (c)	37207	9.5.33	12	6	13809	Bluetongue (characterised by cyanosis and early stages necrosis lips and tongue)	Swelling, cyanosis and early necrotic foci tongue, lips, soft palate; wellmarked hyperaemia and oedema lungs; hyperaemia nasal mucosa; localised hyperaemia rumen, reticulum and omasum; multiple ulcers pylorus; few ecchymoses small intestine; hyperaemia large intestine; numerous parasitic nodules in intestines; hyperaemia larynx and trachea; haemorrhages myocardium	*Lips*: Around root of hairs haemorrhagic zone. *Lip* (i) *Skin*: "ballooning" of epithelial cells in stratum spinosum with extensive infiltration of neutrophiles; earlier lesions show extensive infiltration of corium with blood and desquamation of necrotic parts, involving almost complete loss of the epithelial layers. (ii) *Mucous Membrane*: haemorrhages in the corium without and with neutrophilic infiltrations, extending into the epithelial layers. No specific changes in kidneys, myocardium and lungs.

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2. *Experimental Sheep Cases*—(contd.).

No. of Expt.	No. of Sheep.	Death. Date.	Death. No. of Days after Injection.	No. of Days of Reaction.	Specimen No.	Etiology.	Macroscopical.	Microscopical.
1 (c)	37297	11.5.33	14	8	13860	Bluetongue (characterised by hydropericard, marked hyperaemia and oedema lungs, extravasations epicard and endocard)	Hydropericard ; marked hyperaemia and oedema lungs ; extravasations epicard and endocard ; general cyanosis ; venous hyperaemia liver, kidneys and spleen ; hyperaemia upper extremities hoofs ; hyperaemia margin lower lip ; slight acute catarrhal enteritis ; hyperaemia pharyngeal and parapharyngeal lymph glands and papillae rumen : localised slight hyperaemia pyloris	In sections from myocardium and tongue no specific changes except hyperaemia and haemorrhages.
1 (c)	37280	23.5.33	27	20	—	Pneumonia..........	Advanced post-mortem changes ; marked extravasations conjunctiva (right eye) ; oedema of lungs ; acute bronchopneumonia ; localised fibrinous pleuritis	No material.
2	37452	10.5.33	14	11	—	Bluetongue (oedema of the glottis) (characterised by extensive transudation into connective tissues)	General anaemia and emaciation ; slight post-mortem changes ; oedema of subcutis of cervical region ; healing out ulcers inside of cheek ; severe oedema of glottis ; multiple haemorrhages on pharyngeal muscles ; hyperaemia and oedema lungs (marked) ; hydrothorax ; few *Haemonchus contortus* and *Oesophagstomum columbianum. (Cysticercus tenuicollis* in abdominal and pelvic cavity ; slight hydropericardium ; *Oestrus ovis* larvae	,,
2	37302	27.5.33	22	13	13928	Bluetongue (characterised by hydropericard, oedema and atelectasis lungs)	Well - marked hydropericard, haemorrhages myocard ; well-marked oedema and slight hyperaemia lungs ; atelectasis both lungs resulting from bronchitis ; hyperaemia and degeneration liver ; hyperaemia kidneys ; slight hyperaemia spleen and intestines	*Lung* : Oedema and hyperaemia. *Myocardium.* Slight hyperaemia and haemorrhages endocardium.

2	37454	15.5.33	9	5	13872	Bluetongue (characterised by oedema lungs, necrosis tongue and haemorrhages mouth, heart, etc.)	Hyperaemia coronets; slight ecchymoses skin (under tail); hyperaemia and necrosis lips; subepicard, endocard, and myocardial petechiae and ecchymoses; very marked oedema lungs; necrosis angle of mouth, localised necrosis hard palate; necrosis ventral and lateral aspect of tongue; hyperaemia pharynx and larynx; hyperaemia and necrosis anti and oesophageal groove; haemorrhagic rumen gastritis and enteritis	Lip (i) Skin: Haemorrhages in the form of a zone around the root of the hair in its follicle; here and there necrosis of epithelium and infiltration of the epithelial layers with neutrophiles, much less changes in the corium; irregular loss of some of these infiltrated areas, involving the greater part of the epithelial layers; the different stages are not well defined; at margin of skin and mucous membrane this loss involves a fairly large area with haemorrhage and slight infiltration of the underlying tissues; rest of the mucous membrane of the lip shows no changes. Oesophagus: Haemorrhages into the corium of a few papillae. Tongue: Fairly extensive ulceration of mucous membrane; the base of the ulcer being formed by irregular remains of epithelial cells in the stage of necrobiosis, with haemorrhage and infiltration of the underlying corium, but the latter is slight in comparison with the infiltration in the epithelial layers; the process is primarily associated with disturbances in the epithelial layers and of an acute nature without evidence of proliferation of connective tissue. In another section of the tongue there is "ballooning" of a few cells in the stratum granulosum, the cells being swollen, but spherical and their cytoplasm appears homogeneous glassy-looking and of a light brick red colour with eosin; the nuclei appear "ghost-like". Myocardium: Shows extensive haemorrhages.
2	37679	24.5.33	17	10	—	Broncho-pneumonia; sequel to aspiration of ingesta during bluetongue reaction	Post-mortem changes; general cyanosis; degenerative changes liver; acute catarrhal broncho-pneumonia; fibrinous pleuritis; degenerative changes kidney; slight chronic catarrhal cholecystitis; slight nodular oesophagostomiasis; dilatation heart; tumor splenis; hyperaemia lymph glands respiratory tract	No material.

2. *Experimental Sheep Cases*—(contd.).

No. of Expt.	No. of Sheep.	Death. Date.	Death. No. of Days after Injection.	No. of Days of Reaction.	Specimen No.	Etiology.	Macroscopical.	Microscopical.
2	37691	26.5.33	12	2	13922	Killed *in extremis*, bluetongue (characterised by necrotic lesions and haemorrhages)	Ulceration of lips and buccal mucosa; haemorrhages lateral border tongue; oedema lips, intermandibular spaces, subcutis neck, larynx and pharynx; ecchymoses small intestines; ulceration pyloris; subepicardial and endocardial haemorrhages; hyperaemia lungs; parasitic nodules intestines; degeneration liver and kidneys	*Lips:* Here and there haemorrhages into the corium of the papillae. *Small Intestines:* haemorrhages here and there between the glands extending into the connective tissue. *Myocardium:* Extensive multiple haemorrhages, with well-defined patches of fatty changes. *Lung:* Multiple haemorrhages. *Liver:* No specific changes.
2	37810	26.5.33	10	4	13921	Bluetongue (characterised by multiple haemorrhages and oedema of the subcutis)	Very slight post-mortem changes; slight general anaemia; oedema subcutis of intermandibular space and peri-tracheal tissues; multiple haemorrhages; buccal nasal mucosa; hyperaemia rumen, reticulum and omasum, small intestines, kidneys, liver, and lung; atelectasis lung, haemonchosis; degenerated parasitic nodule liver; caseous nodule lung; extravasations epicard, and right endocardium and to a lesser extent in left endocardium; cestodes, oesophagastomiasis and *Oestrus ovis* larvae	*Lung:* Hyperaemia and small abscess surrounded by granulation tissue. *Tongue:* Haemorrhages in corium of papillae, and in one place localised infiltration of neutrophiles in the stratified epithelium. *Myocardium:* Few haemorrhages in epicardium. *Lung:* Hyperaemia and oedema. *Liver:* haemotoma (probably traumatic). No specific changes in *kidneys*.
3	37165	22.5.33	13	9	—	Bluetongue, (characteristic lesions in mouth disappeared but changes in coronets still present)	Slight haemonchosis; parasitic nodules in intestines; oedema ventral aspect neck; hyperaemia coronets	No material.
3	37768	27.5.33	11	7	13927	Killed *in extremis*, bluetongue (characterised by well-marked oedema of lungs and subcutis and haemorrhages myocard)	Well-marked oedema lungs and slight hyperaemia; fairly extensive oedema of subcutis of intermandibular space, lower cervical region, surrounding tissue of trachea and pharynx; petechiae epicard; few ecchymoses endocardium and myocardium; hyperaemia and degeneration liver; hyperhaemia kidneys; slight tumor splenis; slight hyperaemia oesophageal groove; ulceration pyloris; slight acute haemorrhagic enteritis; oedema and haemorrhages lips and tongue.	*Lips:* Haemorrhages into corium mainly of the papillae; in one place the stratified epithelium infiltrated with neutrophiles which extends on to the corium. *Heart:* Haemorrhages epicardium extending into the myocardium. No specific lesions in *liver, kidneys, lungs, spleen, adrenals, brain*.

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						Diagnosis	Post-mortem	
9	37168	8.5.33	11	8	—	Bluetongue (characterised by oedema)	Oedema subcutis head; oedema lungs; marked oedema tongue, glottis and peritracheal tissues; petechiae abomasum and small intestines; subepicardial petechiae; slight tumor splenis	No material.
9	37089	24.5.33	—	—	—	Bluetongue (characterised by necrosis and extravasations epicard, endocard, bladder, and intestines)	Superficial necrosis external nares, mouth, and inside cheeks; marked petechiae and ecchymoses endocard; hydropericard; localised superficial necrosis ventral aspect tongue; acute haemorrhagic enteritis; petechiae mucosa bladder and urethra	::
9	37079	25.5.33	14	8	—	Bluetongue (characterised by slight necrosis tongue, oedema lungs, glottis, etc.)	Post-mortem changes fairly advanced; marked hydropericardium; hyperaemia and necrosis tip of tongue; oedema glottis and slight oedema intermandibular space; marked oedema lungs; subendocard and epicard haemorrhages; acute haemorrhagic gastro-enteritis; localised hyperaemia urethra; ecchymoses urinary bladder; hyperaemia coronets	,,
9	37086	22.5.33	11	6	—	Bluetongue (characterised by extensive necrotic changes mouth and slight oedema lungs)	Post-mortem changes advanced; excoriations external nares; mandibular hyperaemia lymphatic gland, necrosis buccal mucosa, especially inside lower lips and tongue; localised urethritis; hyperaemia small intestine; haemonchosis; slight oedema lungs; hydropericardium; cyanosis skin	*Parapharyngeal lymph glands*: Revealed no specific changes.
9	37078	23.5.33	12	7	—	Bluetongue (characterised by oedema, necrosis external nares and angle of mouth and extensive oedema) *Note.*—complicated with peritonitis as result of rectal perforation.	Marked oedema subcutis along ventral aspect neck intermandibular space, head, supra-obited fassae, ears and upper portions of fore limbs; acute purulent peritonitis with ascetis; marked hydrothorax; fairly marked hydropericardium; few endocardial petechiae; necrosis external nares, medial septum of nose and angles of mouth; haemonchosis; fistula rectum (perforation by thermometer)	*Lungs*: Slight hyperaemia. *Myocardium*: Slight hyperaemia and in places fatty changes.

2. *Experimental Sheep Cases*—(contd.).

No. of Expt.	No. of Sheep.	Death. Date.	Death. No. of Days after Injection.	No. of Days of Reaction.	Specimen No.	Etiology.	Macroscopical.	Microscopical.
10 (a)	36812	8.6.33	13	6	13961	Killed *in extremis*, bluetongue (characterised by necrotic changes tongue, oedema lungs, etc.)	General oedema; ulcerative glossitis; severe pulmonary oedema; extravasations left endocardium and pulmonary artery; hyperaemia fore-stomachs and small intestine	*Pulmonary artery*: haemorrhages into media-adventitia. *Tongue*: Classical case in which all the lesions occurred in various stages:— *1st stage*: Hyaline droplet-like formation in swollen epithelial cells in the stratified epithelium near the basal membrane, some of the epithelial cells show ballooning, but their contour is not lost. *2nd stage*: the vacuolated spaces in the stratified epithelium infiltrated with neutrophiles, not many neutrophiles in these spaces. *3rd stage*: Necrobiosis of the epithelial cells and neutrophilic infiltration into the stratified epithelium. The corium bordering on this shows haemorrhages and extensive infiltrations with neutrophiles. *4th stage*: Partial desquamation of the necrotic areas with the whole contour of the mucous membrane disorganised as a result of necrotic material and infiltrated cells.
10 (a)	36779	12.7.33	6	4	14065	Killed in early stages of bluetongue for collection of material (characterised by multiple haemorrhages of mucous membrane of tongue and extensive oedema of subcutis)	Well-marked oedema in intermandibular space, extending on to the masseters and ears; multiple haemorrhages of mucous membranes of lips, tongue epicardium, right endocardium, larynx, subcutis, skin, omasum and abomasum; slight degenerative changes kidney and liver; haemorrhage pulmonary artery above the semilunar valves	*Tongue*: Extensive circumscribed haemorrhages into epithelial layers extending from basal membrane to the flattened superficial layers, with atrophy of adjoining epithelial cells; the underlying corium shows practically no changes. *Pulmonary artery*: extensive haemorrhage into the media and adventitia. *Epicardium (at the apex)*: Fairly extensive haemorrhage extending into the myocardium. *Spleen, liver, and kidneys*: No specific lesions.
10 (b)	37806	—	12	8	14104	Bluetongue (characterised by subcutis oedema and localised necrotic lesions in various organs in process of healing out)	Fairly marked oedema of subcutis, peritracheal perioesophageal connective tissues, oedema of the lungs, hyperaemia of corium of hoofs, subepicardial and subendocardial haemorrhages, erosions and swelling tongue and lips, hyperaemia and superficial erosions mucous membrane of rumen, reticulum and omasum; excoriations skin and vagina	*Vulva*: Fairly. Extensive ulceration of the mucous membrane, the base of the ulcer being formed by the corium which is very extensively infiltrated with neutrophiles, these extend into the depth of the corium to a considerable extent, the cells involved show necrobiosis in places.

10 (b)	36976	4.7.33	15	10	14051	Killed in extremis, bluetongue (characterised by marked prostration)	Emaciation, discharge of ingesta through the nose; subendocardial haemorrhages and haemorrhages under capsule of the spleen	No material.
10 (c)	37801	17.7.33	10	7	14078	Bluetongue (characterised by multiple haemorrhages)	Petechiae and ecchymoses, mucous membranes, tongue, cheeks and lips; subepicardial and endocardial haemorrhages; slight oedema of the lungs, swelling and petechiae of reticulum and oesophageal groove; acute haemorrhagic enteritis with numerous small petechia, mucous membrane intestines; petechia bladder and skin in vicinity of mammae and joints; oedema of subcutis of ventral aspect cervical region	Tongue: Here and there haemorrhages into the corium of the papillae Myocardium: Multiple haemorrhages not frequent and not not extensive, in places associated with infiltration with neutrophiles. All other organs examined show no specific changes.
10 (c)	37780	29.7.33	11	7	14105	Killed in extremis, bluetongue (characterised by extensive oedema of subcutis and petechiae and ecchymoses in various organs)	Extensive oedema subcutis peritracheal and perioesophageal connective tissues; oedema of lungs and tongue; subepicardial and endocardial haemorrhages; excoriations vagina and nostrils; localised hyperaemia rumen, reticulum and omasum, petechiae abomasum and small intestines	Rumen: Musculature shows multiple haemorrhages. Other organs show no specific changes.
10 (c)	37782	25.7.33	8	6	14096	Bluetongue (characterised by oedematous and petechiae)	Oedema of the subcutis, subepicardial and subendocardial haemorrhages; slight oedema of the lungs, oedema of peritracheal tissues, larynx and tongue; petechiae ventral aspect of tongue; oedema of lips with petechiae on the mucous membrane; haemorrhages papillae of rumen, reticulum and omasum; localised hyperaemia pylorus	Spleen, kidneys, liver, myocardium show no specific changes. Rumen: Some of the papillae show fairly characteristic changes, viz., "ballooning" of the stratified epithelial cells, corresponding more or less with "stratum granulosum"; this "ballooning" is characterised by large spherical appearance of the epithelial cells, some of them showing the presence of a filamentous-like material of a light pink colour with eosin; in others this material is more compact and homogenous; in the majority of affected cells there is loss of nuclei, in places these enlarged cells show infiltration with neutrophiles. in some the contour of the cells still well defined. Except for haemorrhages in the papillae of the corium it is remarkable how free it is of any inflitrative changes.

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APPENDIX C.

(1) Extracts from Reports by Veterinary Officers on the Occurrence of this Bluetongue in Cattle.

Note: That the veterinary officers were dealing with bluetongue in cattle, is based upon the descriptions given in field reports. No aetiological diagnosis is given in these reports, and apparently the disease was no recognised as bluetongue. In some instances the descriptions are not sufficiently detailed, and the conclusion of the existence of bluetongue is not definite. It should be remembered that an investigation of these outbreaks was primarily undertaken to exclude foot and mouth disease and not to ascertain the nature of the disease.

Date and Locality.	Report by.	Extract in Field Reports.
24.2.33, Vlakfontein, Nigel, Tvl.	T. Adelaar.........	" 1 *ox.* Necrosis on the dental pad."
23.2.33, Paardefontein, Heidelberg, Tvl.	D. G. Steyn.......	" 1 *ox.* Animal listless. Mucopurulent discharge from the nose. Ulceration of interdigital space. Extensive ulceration of mucous membrane of upper and lower lips, gums and dental pad."
27.2.33, Klipriver, Ladysmith, Natal	J. R. Frean........	" 1 *calf.* Salivation and stiff gait. Small ulcers on upper gum. Diphtheritic deposit ventral surface of the tongue."
28.2.33, Trumpeters Post, Mafeking	J. J. Keppel.......	" *Calves* (the number affected not stated). The calves show peculiar lesions on the dental pad.` These lesions are ephemeral, and as rapidly as they appear they disappear, leaving a small circular lesion without ulceration. These small lesions are multiple and close together."
3.3.33, Townlands, Standerton, Tvl.	J. G. Bekker.......	" 1 *cow.* Superficial diffuse necrosis on muzzle, inside of nostrils, dental pad and gingiva. Hyperaemia of the tongue. Teats all sore. Calf of cow normal. Temperature varied at different examinations 106° to 101°. Later marked skin lesions developed."
16.3.33, 5.3.33, 9.3.33, different farms in Mafeking and Marico districts	N. C. Starke.......	" *Number of calves* " the condition noted in these calves is referred to as a " diphtheritic stomatitis."
5.3.33, Oudehoutsdraai, Volksrust	J. Dickson and J. G. Williams	" 2 *cattle* " (sex not given). " Two animals showed necrotic lesions in the mouths. In one animal the tongue was protruding about 8 inches. The organ was bluish discoloured and was swollen and firm. Small necrotic foci were also evident on the under surface of the tongue. There were also other cattle on the farm affected with stiffsickness, but apart from the stiffness nothing abnormal and no mouth lesions were present."

Date and Locality.	Report by.	Extract in Field Reports.
12.3.33, Blinkpoort, Heidelberg	J. B. Quinlan......	"*One cow.* Animal *in extremis.* Necrosis ventral aspect of tongue and on the dental pad."
14.3.33, Vlakplaas, Germiston, Tvl.	J. B. Quinlan......	"*One heifer.* Superficial ulceration of the upper and lower lips and superficial ulceration on the ventral aspect of the tongue. In the region of the fetlocks the skin felt hot and was very red. The interdigital space showed epithelial excoriation."
14.3.33, Hartlam, Reitz, O.F.S.	C. C. Wessels......	"*One ox.* Profuse salivation and mucopurulent discharge from nostrils. Several abrasions on the mucosa of the dental pad, lips and underface of the tongue. The lower lip was also slightly swollen. Some of the papillae also showed hyperaemia and were swollen. On examination of the nasal cavities there were two small ulcers on the septum nasi."
17.3.33, Poortjiesfontein, Standerton, Tvl.	J. G. Bekker......	"*Two milch cows.* Temperatures 103·5 and 104·5·. Necrosis on the dental pad, upper and lower lips and underneath the tongue. Distinct swelling of the lower portions of the limbs and redness on the plantar aspect of the digits. The teats were sore and an exudative inflamation of the skin of the flanks noted."
18.3.33, Kromdraai, Standerton, Tvl.	J. G. Bekker......	"*One milch cow.* This animal was frequently examined and showed necrosis of lips, gums and ventral aspect of tongue. Teats sore. Later extensive skin lesions developed."
14.3.33, Welgezegend, Standerton, Tvl.	J. B. Quinlan and J. G. Bekker	"*3 cows.* Temperatures 103, 104, and 104·5 Necrosis dental pad, upper and lower lips, muzzle and behind incisor teeth. In one case the tongue was protruding and swollen. On the ventral aspect of apex the mucosa appears raw and very red. The tongue of another beast also severely affected and a large deep seated necrotic lesion on lateral aspect in the region of the body of this organ. The lower portions of the limbs in all cases appeared swollen and the surface of the skin in the interdigital spaces appeared moist and excoriated. All the udders were affected, especially the teats, which were red and covered with thin crusts." *Note.*—A fuller description of these cases is given in Appendix " A."

Date and Locality.	Report by.	Extracts in Field Reports.
19.3.33, Rooikop, Germiston, Tvl.	P. S. Snyman......	" 1 *ox.* Necrosis lips, dental pad, etc."
20.3.33, Ascent, Vrede, O.F.S.	J. B. Quinlan and J. G. Bekker	" 1 *ox.* Swelling in the region of fetlocks. Necrosis of anterior aspect of dental pad and borders of lips."
27.3.33, Verblyden, Standerton, Tvl.	J. G. Bekker......	" 1 *heifer.* Temperature 106°. Necrosis dental pad and lips. The papillae on lips swollen and tips yellow in colour (necrosis), bases red. Feet swollen and an anterior aspect of interdigital spaces triangular areas, which are covered with dark brown scabs and underneath a raw granulating surface. Distinct ' break ' at the coronets."
29.3.33, Mietjiesfontein and Rietvlei, in the Koedoesrand, N.Tvl.	G. de Kock........	Describes a condition seen in cattle and he refers to it in the following way : " Cattle were seen showing a disease condition resembling three-day stiffsickness. In two cases I was struck with the swelling and redness of the tongues."
1.4.33, Swartland, N.Tvl......	P. S. Snyman......	Describes a cattle disease on this farm as follows : " The lesions in the mouth begin as a small swelling in the form of a pimple. Necrosis of the mucosa in the region of the swelling soon takes place and enlarges rapidly. This is seen especially on the ventral aspect of the tongue. No lesions were observed in the interdigital spaces, but lesions were present on the anterior aspects of the coronets. These showed a tendency to spread to the plantar aspects. One case was encountered with lesions of the skin of the udder and flanks. The epidermis was red and could easily be removed leaving a moist surface."

(2) General Reports on this Disease.

The following interesting and valuable information on this disease of cattle has come to our notice :—

Mr. F. A. Verney, Principal Veterinary Officer, Basutoland, in his annual report of the year 1932, states :—

" In the autumn a cattle disease occurred that simulated very closely all the symptoms of foot and mouth disease, and had I not observed this disease 16 years ago, I should have been genuinely alarmed. The disease is indicated by an ulcerative stomatitis, loss of appetite, rise in body temperature, usually associated with a stiff gait and swollen fetlocks, occasionally with necrotic sores in the interdigital space. The disease usually breaks out in individuals in a herd, and shows no evidence of being infective and all my efforts to infect other cattle with saliva proved futile, and it is this factor that makes one definitely to decide against the dreaded contagious foot and mouth disease."

Mr. B. J. Brummer, Government Veterinary Officer, O.F.S., in a report in March, 1933, states :—

" I have seen sporadic cases of this disease in various parts of the Free State. Generally only one or two cases occur on a farm . . . . The most common lesions seen are : an acute dermatitis of the skin around the mouth. The skin is just inflamed, then gets dry and hard and forms

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cracks all along the borders of the lips. The muzzle becomes dry and painful, and the animal will often lick this part with the tongue. A burning sensation must be present, as the animal often shakes its head and even becomes vicious in the acute stages. Later the superficial layers of the skin peel off. In severe cases, the mucous membrane of the mouth is also inflamed and red. This may pass off without any further lesions, but usually lesions resembling that of *bluetongue in sheep* develop. These are of a diffuse nature and are mostly formed on the dental pad, along the borders of the lips and sometimes under the tongue. The mucosa of the nostrils is also generally inflamed, very sensitive, and easily bleeds.

"In most cases an acute laminitis is present in all four feet, accompanied by a redness of the skin along the coronets and a swelling of the lower parts of the legs. The swelling is most noticeable around the fetlock joint and may extend up to the knees and hocks, but I have never seen any sores formed (unless of a traumatic nature) along the coronets and between the claws.

"In fatal cases death occurs early in the acute stages before the lesions become properly developed. Once the skin lesions are noticeable, the animal is usually past the danger point. Generally a slight gastro-enteritis is also present. The disease occurs very sporadically and leaves little opportunity for further investigation."

Mr. Brummer suspected a poisonous plant as a possible cause of this disease.

Mr. C. C. Wessels, Government Veterinary Officer, Bloemfontein, in a general report on this disease, states that during April and March, 1933, numerous outbreaks of this disease occurred in various parts of the Free State. He mentions that when one of these cases occurred, all the neighbouring farmers were greatly perturbed since they suspected foot and mouth disease.

Mr. S. Reynolds (farmer), Zandbaken, Val, Standerton, in a letter dated 19th March, 1933, to the Director of Veterinary Services, referred to the disease as follows:—

"I find that the disease is to a certain extent seasonal, and mostly, if not always, only noticed during March and April. It bears many similarities to bluetongue of sheep and being most acute—as with most other South African diseases—in cattle imported from Europe. The affected cattle run a high temperature with complete or partial loss of use of the tongue. The tongue often going dark blue in colour with inflammation and eruption of skin around and under the tongue and lips. The feet are tender and sore and the animals move stiffly; in other cases, no feet disturbances are noted. In more acute cases—as it varies, of course, in intensity—new hoofs would grow through and replace the old ones. The disease lasts a few days and then recovery would be rapid."

In a letter, dated 19th March, 1933, to the Director of Veterinary Services, *Mr. Armitage* (farmer), of Vaalbank, Standerton, describes a condition encountered in 1931 in two of his cattle (a year-old-bull and a two-year-old heifer) in the following way:—

"Both cases were very similar and started with high temperatures within a day of each other. The animals were very stiff and lame; in fact, a few days later they could hardly stand. The lower portions of the limbs were sore and swollen, but no open sores were seen. Two or three days later the tongues became very swollen and pinkish blue in colour. They seemed quite paralysed and hung out of the mouths. A hard scale formed over the upper lips and nostrils. This scale gradually became loose and fell off in one piece as the animals got better.

"The affected cattle could not eat for some time, but appeared to be hungry and were fed with slices of mangels and pumpkins, which were placed on the back of the tongues.

"As the condition improved the animals began feeding again, but were stiff for some considerable time.

"The heifer was turned out to graze as soon as she could walk. The young bull was left in with my other bulls, and I noticed sometime afterwards that all his hoofs were very long and found that new ones were growing. Some time later the old hoofs fell off."

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APPENDIX D.

TEMPERATURE CHARTS OF EXPERIMENTALLY INFECTED SHEEP AND CALVES.
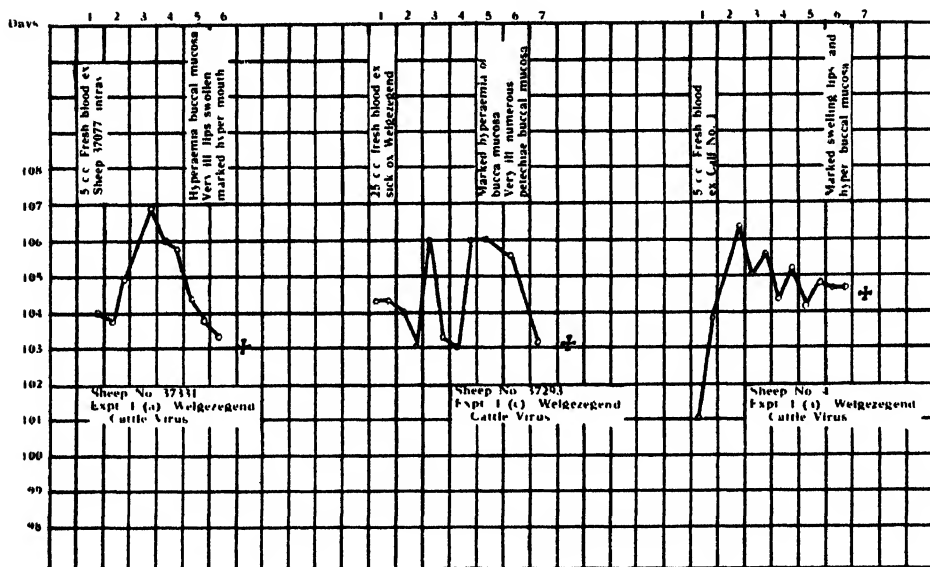
CHART I.

Peracute reactions.
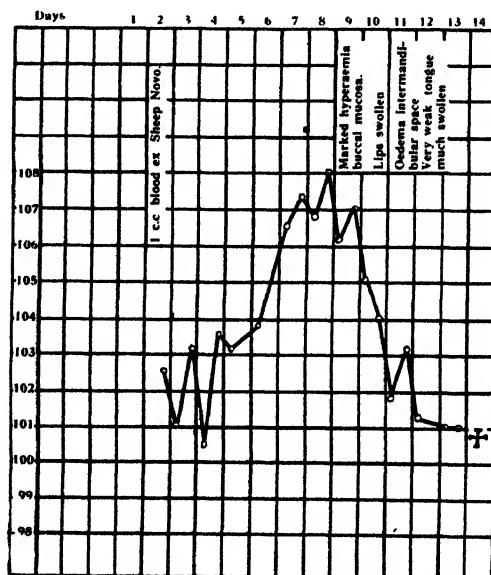
CHART II.

Sheep 37078, Expt. 9. Acute reaction. NOTE: Very high temperature and collapse.

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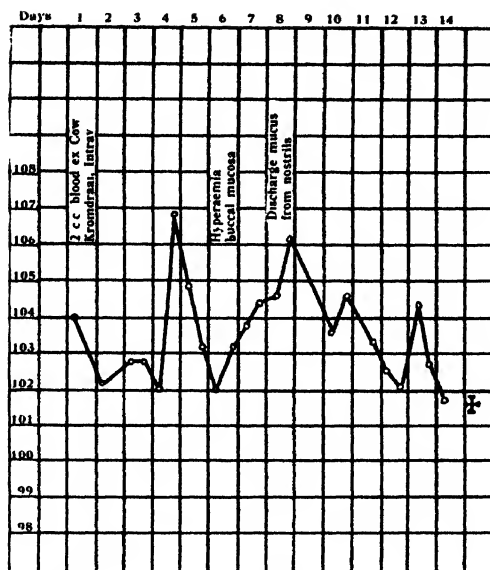
CHART III.

Sheep 37452, Expt. 2. Acute reaction. NOTE: Intermittent nature of temperature.
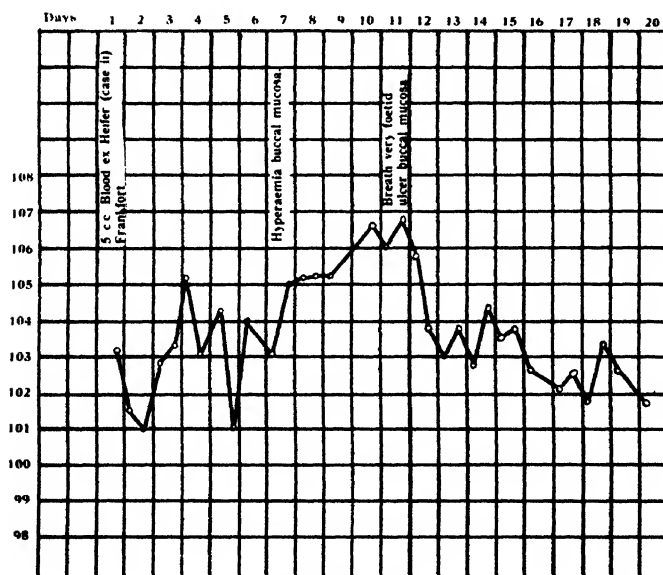
CHART IV.

Sheep 37678, Expt. 7 (b). Acute reaction. NOTE: Long duration of temperature.

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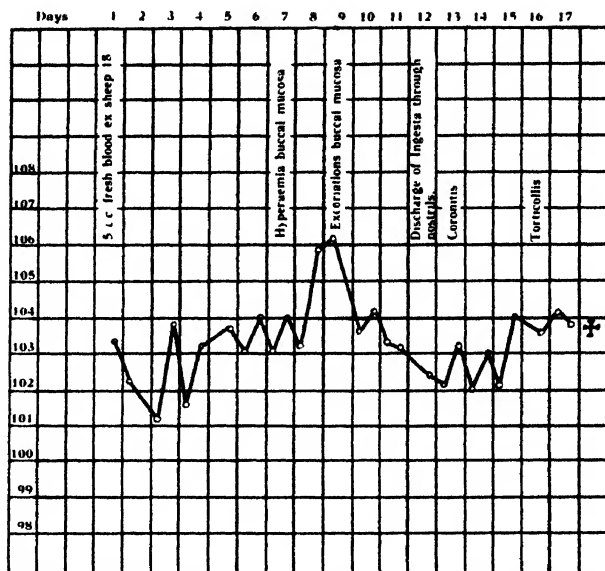
CHART V.

Sheep 37679, Expt. 2. Subacute reaction. NOTE: Short duration of temperature reaction (ephemeral).
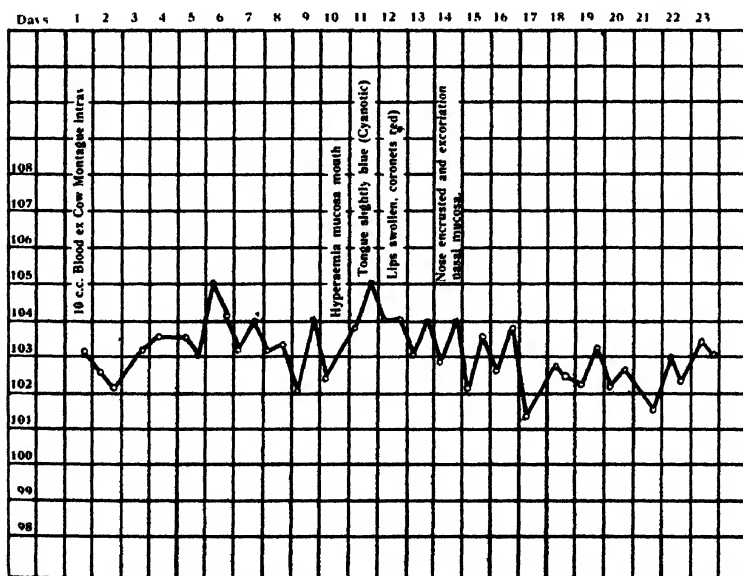
CHART VI.

Sheep 37174, Expt. 6. NOTE: Slight elevation temperature (a febrile reaction), but distinct development of lesions.
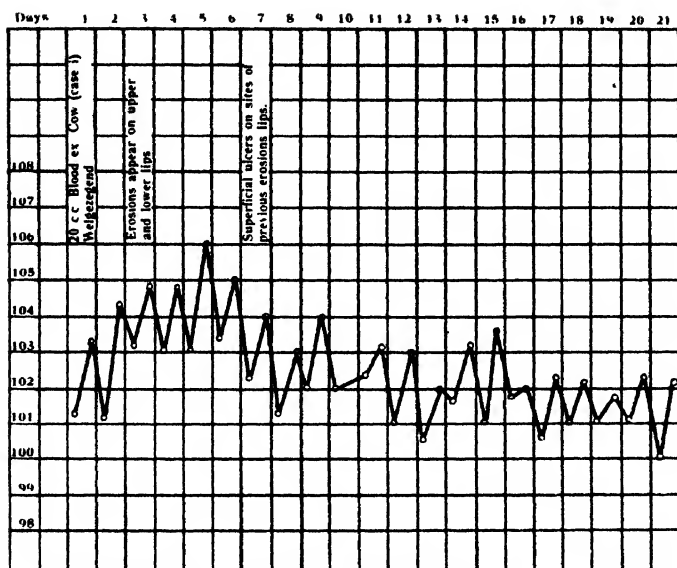
CHART VII.
Calf 1, Expt. 1 (a).
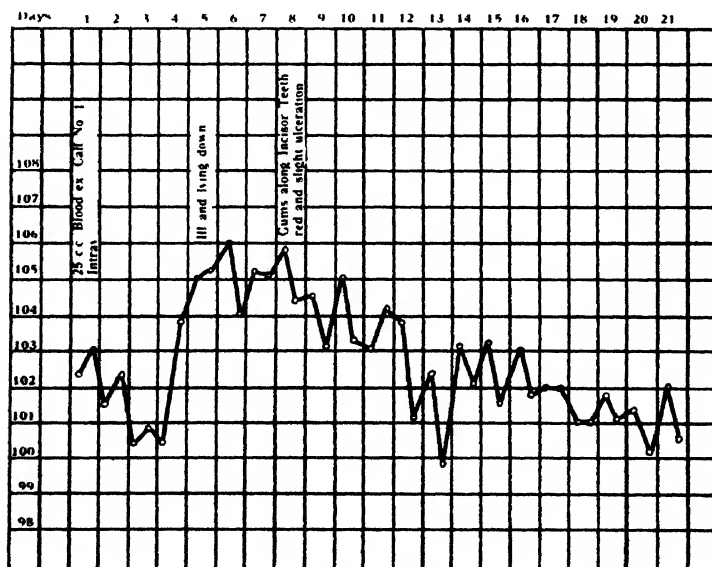
CHART VIII.
Calf 3, Expt. 1 (a).
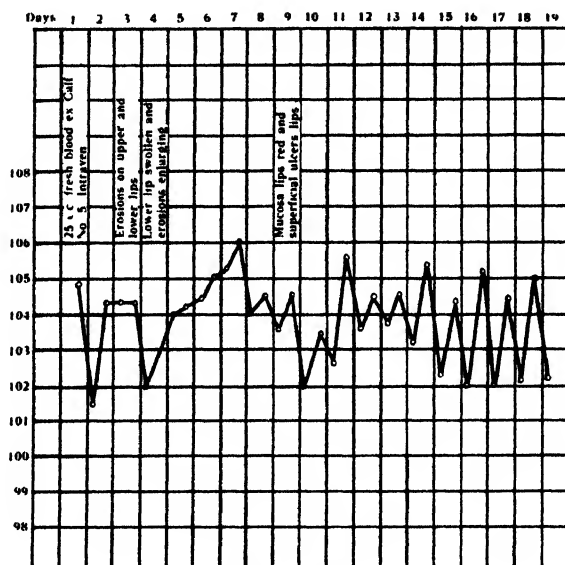
CHART IX.
Calf 10, Expt. 1 (b).
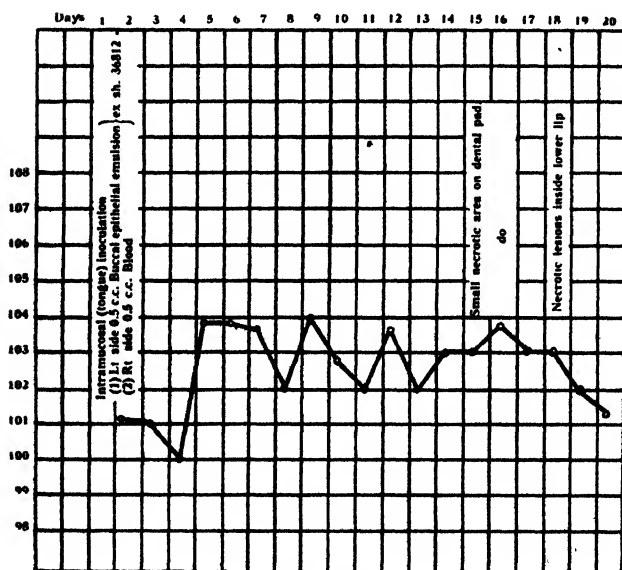
CHART X.
Calf 5201, Expt. 10 (d). NOTE: Temperature recorded once daily,
i.e. at 9 a.m.

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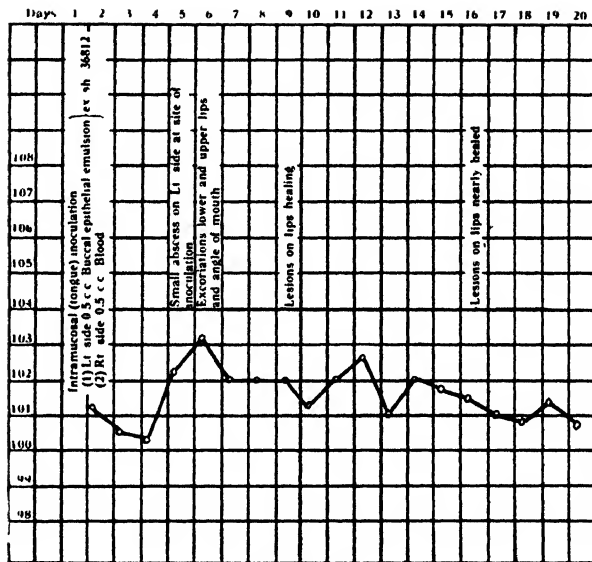
CHART XI.

Calf 5257, Expt. 10 (d). NOTE: Temperature recorded once daily, i.e. 9 a.m.

Investigations into the Transmission of Bluetongue in Sheep during the Season 1931/1932.

By

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IN addition to the work on horsesickness described in the preceding paper, we carried out a number of experiments with bluetongue in sheep along similar lines. The work on bluetongue was mainly undertaken to make use of the time, when the horsesickness work could not be undertaken.

From the epizootological point of view, horsesickness and bluetongue are very closely related to one another, and what has been said about horsesickness—it being non-contagious by ordinary means of contact, the seasonal and also to a certain extent the geographical distribution, the dependence on the amount of rain, the occurrence in marshy vleis and absence on hill tops, the danger of grazing at night, during the late afternoon or the early hours of the morning, the protection afforded by stables—applies practically as well to horsesickness as to bluetongue and does not need to be discussed at length.

It seems to be very probable from the information at hand, that horsesickness and bluetongue, if transmitted at all through insects, are carried by the same or by closely related species. As in the case of horsesickness we suspected, for reasons discussed with the results of the mosquito survey, *Aedes* species as the most probable transmitting agents, and this genus only was used in our experiments.

According to references in the literature up to now, no transmission experiments have been carried out with bluetongue.

I. EXPERIMENTAL TECHNIQUE.

The methods adopted in our work on bluetongue were in general very similar to those used for horsesickness, and have been described in full in the second paper of this series. We can limit ourselves therefore to a brief summary.

The mosquitos were obtained by catching the adults and larvae or pupae in the field and keeping the latter in the laboratory until the imagines hatched out. The larvae originated from natural breeding places, or during the drier part of the season from artificially flooded places. Specimens hatched in the laboratory from larvae or pupae obtained from the field were more suitable than those caught as adults, the latter generally showing a much higher mortality.

The mosquitoes were kept, before use, in large mosquito netting enclosed cages covered on four sides by hessian kept wet by allowing water to percolate through it from above, in order to secure the necessary degree of humidity. When not needed directly they were fed on a 10 per cent. aqueous solution of sugar, otherwise pure water only was provided.

The infected specimens were stored in our warm room, in which the temperature was maintained at an average of 24° to 25° C. From time to time, however, the temperature varied between 20° and 30° C. as the heating had to be controlled by hand.

In the first series of experiments the mosquitoes were all stored in jam jars, the lids being replaced by mosquito netting, 10 specimens in each. In order to secure the necessary humidity the jars were kept on wet cotton wool in slightly larger jars provided with lids having a few holes in them. The mosquitoes were fed on sugar water, a small piece of cotton wool soaked in a 10 per cent. solution being placed on the net covering the jar. This method was quite satisfactory, as was the case with horsesickness, when the mosquitoes were used for injection and only small numbers needed.

For the experiment in which the feeding of larger numbers was intended, our specially designed cages were employed. These were kept on shelves, being surrounded on four sides by wet hessian, thus ensuring the desired humid conditions inside the cages. For some species, especially *A. caballus*, it was found advisable to further increase the humidity by covering the floors of the cages with wet filter paper. In the case of the small mosquito groups the jars were always employed, as the cages, which were limited in number, had to be reserved for the larger groups.

The method employed for the injection of mosquitoes consisted firstly of stunning the insects by hitting the test tubes in which they were contained against the palm of the hand. The stunned specimens were then dropped on to a small quantity of normal sheep serum contained in a mortar and ground up to form a fine emulsion by means of a pestel. A further quantity of serum was then added and the whole injected subcutaneously into the sheep on the inner aspect of one thigh.

For feeding purposes the small wire cages covered with mosquito netting, described in the technical section, were used in all cases. The liberating of mosquitoes in fly-proof stables containing sheep was not attempted, as no good results were expected.

The wool was carefully clipped as close as possible to the skin from an area on the back of the sheep corresponding with one surface

of the cage, and the cage, containing the mosquitoes, was then placed in position on this area. Six pieces of tape attached to the wool around the clipped area, two on each of the long sides and one on each of the short ends, tied together over the cage served to hold it in position. To ensure a sufficient degree of humidity inside the cages a piece of damp cotton wool was placed on top of the cage and held in position by means of the tapes. Without this precaution a considerable mortality could occur. Up to four cages could be placed on one sheep in this manner, two on either side of the mid line.

As our stable for mosquito feeding, with special arrangements for obtaining a humid atmosphere, was mostly in use for horsesickness experiments, the feeding on sheep had to be conducted in the ordinary sheep stables. On very dry nights the cotton wool occasionally dried up, resulting in a more or less high mortality amongst the mosquitoes, but under normal conditions the water contained in the cotton wool was sufficient to last out the night.

By this method of mosquito feeding it was not necessary to isolate individual sheep, as the cages are not interfered with by other sheep which may be present in the same box. Furthermore, the sheep used were apparently in no way irritated by the presence of the cage(s), as no attempt was made to rub it (or them) off, or, at any rate in our experiments no sheep succeeded in dislodging a cage.

The feeding results on the whole were not as good as was the case in the horsesickness experiments. The percentage of mosquitoes which engorged themselves, and furthermore, the amount of blood taken up by the individuals was definitely less, whereas the mortality amongst the unfed specimens was certainly higher. A number of reasons may be advanced to account for this fact. The pressure exerted on the cage, on which depended its contact with the skin, was not so great as in the case of the cages used on horses, so that slight movements of the skin tended to disturb feeding mosquitoes. The regulation of humidity was less effective and the mosquitoes had ample opportunity of taking up water. Short wool covering the clipped surface, which was never shaved, mechanically interfered with the insertion of the proboscis and the wool fat present probably also played a part in preventing good feeding.

However, our results were sufficiently satisfactory to permit of our disregarding these minor difficulties.

II. STRAINS OF VIRUS USED IN THE EXPERIMENTS AND EXPERIMENTAL ANIMALS.

In our experiments three different strains of bluetongue virus were used, the ordinary vaccine strain, and two strains recovered from fresh spontaneous cases.

1. *Bluetongue Vaccine Virus.*—In the first series of experiments described hereafter the virus wherewith we produced the experimental cases of bluetongue for feeding mosquitoes on, consisted of the laboratory vaccine strain. This strain had originally been obtained from a natural case of bluetongue which had occurred on the station in a lamb in February, 1927. Blood from this lamb had been

injected into sheep from which, by successive subinoculations into other sheep at or immediately after the reaction period, the virus strain. had passed through 48 generations. These subinoculations were carried out over the period 5.2.27 to March, 1932, the last sheep being 31930 and 31554, the preserved blood of which was used in our experiments.

On reviewing the history of this virus strain certain facts are apparent which would account for some of our failures or apparent failures to transmit the disease through the agency of mosquitoes. In the first place only two deaths, which could be definitely attributed to blue tongue, were caused. Of the two sheep injected with blood from the original lamb which died of bluetongue, one died on the fourth day after injection after having shown no temperature reaction whatsoever, the other showed a typical though not marked reaction commncing on the sixth day and returning to normal on the eleventh day, the maximum temperature registered being 105.2° F. This latter sheep was used for subinoculations into two further sheep, death resulting in both cases with typical symptoms of bluetongue. The first of these two sheep showed a temperature reaction commencing on the third day with a maximum of 106.2° on the seventh day, and death on the ninth day. In the second case the reaction lasted from the seventh to the twelfth days, with a maximum of 105.1°. The temperature then remained in the neighbourhood of 102° F., with death on the seventeenth day. No further deaths were recorded during the whole course of the subinoculations, except five which occurred between the tenth and twenty-second generations, and for which some other explanation, having no relation to bluetongue, could be given.

In all, about 113 sheep were used up to the production of the present vaccine strain. Of these, 86 per cent. showed temperature reactions, 5 per cent. showed very doubtful reactions, whereas about 5 showed no evidences of reactions. Of the 86 per cent. showing reactions 20 per cent. were looked upon as slight reactions, where the temperature remained below 105° and was as a rule not maintained for longer than half a day, or fluctuated markedly between 103° and 105° for a few days. However, the scrutiny of the temperature charts of the sheep used in the production of the virus has revealed the fact that a slight or doubtful reaction does not necessarily indicate the intensity of the reaction following upon a subinoculation of blood from such a case. Marked reactions frequently result from subinoculations from slight cases and the converse also holds good. One further point of interest is the production of clinical symptoms. As pointed out above, only two deaths from bluetongue resulted from subinoculations from the original lamb, both of which occurred in the second generation. Up to the eleventh generation slight mucoid or muco-purulent discharges from the nostrils, dyspnoea, and swelling and reddening of the buccal mucous membrane generally accompanied by reddening of the coronets, were observed. Thereafter, no clinical symptoms were recorded, the virus having become so attenuated as to produce only temperature reactions.

During the course of our work temperature reactions were the only guide as to the success or otherwise of the transmission experiments. In this connection it must be pointed out that only temperatures exceeding 104° F., and maintained for periods of one or more days, and furthermore, making their appearance within the limits of the vaccine strain incubation period, which as revealed during the production of the vaccine varied between three and twelve days, were considered to be positive. There exists, therefore, some doubt as to the possible number of positive cases produced. All the experimental animals were, however, subjected to a further subinoculation of vaccine in order to test their immunity and, although such subinoculations may not always succeed or an animal may have acquired a partial immunity not sufficient to absolutely protect it, this test was looked upon as the final proof of the original transmission of the disease.

2. *Ixopo Virus.*—A sample of blood obtained from a case of bluetongue in sheep on the farm of a Mr. Harrek in the Ixopo district of Natal was received from the Government Veterinary Officer of Ixopo on 4.4.32 and this constituted the virus strain used in our experiments and designated Ixopo virus. Unfortunately no information could be obtained as to the extent and severity of the outbreak, but it was ascertained that sheep had actually died from bluetongue on the farm in question. Bluetongue is known to occur yearly in this locality, so that there can be little doubt but that we were dealing with blood from a naturally contracted case.

3. *Kameelfontein Virus.*—On the 5th April, 1932, the Extension Officer of the Pretoria District sent in blood from three lambs which died of bluetongue belonging to Mr. Opperman of the farm Kameelfontein in this district. Although not a very extensive outbreak, this owner lost several other sheep from typical bluetongue. Yearly inoculation against bluetongue is carried out on this farm, but this was not done this year on account of the season having been very dry and there being little chance of bluetongue in the farmer's opinion. As mentioned previously the blood received was taken from young lambs, so that even if relapses of bluetongue do occur there is little chance of our having obtained anything but a natural field strain of the virus, as this was the first season that these lambs had been exposed to infection.

4. *Experimental Animals.*—The sheep used in our experiments are periodically obtained by the Laboratory for experimental purposes and for the production of the bluetongue vaccine from known bluetongue free areas. They are bought from a few well known reliable farmers in the Phillipstown District of the Cape Province, which is a Karroo area. No guarantee of their susceptibility to bluetongue is obtained, but agreements are made that so far as possible the sheep must be obtained only from the Phillipstown District. A further precaution that is taken is to buy only two-tooth sheep in order that they will have had as little chance as possible of having been exposed to natural infection. It is the absolute exception to encounter an immune sheep, so that for practical purposes it may be concluded that all the sheep used are susceptible to bluetongue.

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III. EXPERIMENTS WITH THE ORDINARY VACCINE STRAIN OF BLUETONGUE VIRUS.

At the commencement of our experiments we only had at our disposal the laboratory strain used for the preparation of the bluetongue vaccine which, as previously stated, had been isolated from a natural case five years before, and had been passed through nearly 50 generations in the laboratory by means of direct successive blood inoculations. This was therefore not the most suitable strain and we could anticipate difficulties in our transmission experiments.

We began the experiments, as in the case of horsesickness, with the injection of infected mosquitoes after intervals varying between ½ and 19 days, dating from the infective meal. In most cases an interval of from 5 to 15 days was chosen. In the event of a positive case being obtained by this method it was our intention to attempt the further transmission of the infection by the feeding of the same species of mosquitoes on susceptible sheep.

The experiments here under review in this section were commenced in November, 1931, and concluded in March, 1932, when we obtained a positive case and at the same time also received material from natural " field " cases. The work was interrupted for more than a month by our attempts to transmit further what we took to be a successful case caused by the original vaccine strain of the virus, but which eventually turned out to be a doubtful temperature reaction.

For these experiments we used six different species of mosquitoes, viz., *Aedes caballus*, *A. dentatus*, *A. hirsutus*, *A. lineatopennis*, *A. punctothoracis* and *A. vittatus*. *Aedes caballus*, *A. lineatopennis* and *A. hirsutus* were regarded as the most probable vectors for reasons outlined above. *Aedes dentatus* and *A. vittatus* were looked upon as of secondary importance, whereas *A. punctothoracis* was not considered to be of any practical significance.

In all, 12 experiments were made in which about 250 mosquitoes were injected into 10 sheep. After an observation period of three weeks or more, these sheep were all injected with 1 to 2 c.c. of bluetongue vaccine (hereafter referred to as B.T.V.) in order to test their susceptibility or immunity. Whenever it was considered necessary blood from the experimental sheep showing a febrile reaction was injected into one or two susceptible animals in order to verify the original reaction.

In the following pages the various experiments themselves will be described and thereafter a discussion of the results of all the experiments together will be given at the end of each section.

(a) VIRUS SHEEP.

Six sheep were used in this series of experiments whereon the mosquitoes were fed. They were injected with the following B.T.V. material: 47th generation (batch 809), 48th generation (sheep 31930) or with blood from one of our own vaccine virus sheep (49th generation).

Virus sheep 1 (=sheep 32230). Injected on 14th November, 1931, with 1 c.c. B.T.V. 31930.

Result.—Temperature normal up to 18th November a.m.; p.m. 105.4°; the following morning 103.8°. Temperature on 20th, 22nd, 23rd, 25th and 27th November between 104° and 104.6°. The temperature reaction was thus not very marked.

With blood from virus sheep 1, two other sheep, viz. 32338 and 31651, were injected. The first sheep showed a typical temperature reaction with 106.9° as maximum. In the case of the second sheep the reaction was weak, the temperature not exceeding 104.7°.

Virus sheep 2 (=sheep 32331) infected in experiment No. 1 (for temperature reaction see experiment 1).

Virus sheep 3 (=sheep 32338) injected on 26th November with 1 c.c. blood from virus sheep 1.

Result.—On 2nd December the temperature began to rise and the febrile reaction lasted for 5 days. On 2nd December the temperature registered 103.8 and 105.6° (morning and afternoon), on the 3rd 105.5 and 104.8°, the 4th 106.5 and 106.9°, the 5th 106.2 and 105.2°, the 6th (a.m.) 104.2° and the 7th 104° and 103.5°. This was looked upon as a medium reaction.

Virus sheep 4 (=sheep 31635). Injected on 27th January, 1932, with 2 c.c. B.T.V. 31930.

Result.—Temperature normal up to the morning of 1st February. Afternoon temperature 104.2°; 2nd (a.m.) 103.3°; 3rd 105.0° and 104.9°; 4th 105.8° and 106.2°; 5th 105.3° and 104°; and 6th and 7th between 104.0° and 105.0°. This was quite a good reaction, lasting 7 days and showing a maximum of 106.2°.

Virus sheep 5 (=sheep 31582). Injected on 18th February with 2 c.c. B.T.V., batch 809, reported by the Government Veterinary Officer, Barberton, as having caused mortality amongst sheep immunized with it under field conditions. In a number of sheep injected with the material at the laboratory the course of the infection was, however, identical to that produced by our ordinary vaccine virus.

Result.—The temperature remained below 104° up to 22nd February. On the 23rd it rose to 104 and 104.4°; the 24th 104 and 104.6°; 25th 105.7 and 106.4; 26th 105.4 and 104.7; 27th 105.0 and 105.3; 28th (a.m.) 105.2°; 29th 106 and 106.3; 1st March 105.0 and 105.4°; 2nd 104.0 a.m. and p.m. and on the 3rd 103.2 and 104.1. Thereafter the temperature remained below 103.6. This temperature reaction was very marked, the maximum being 106.4 and the duration about 10 days.

Virus sheep 6 (=32120). Injected at the same time as virus sheep 5 with the same material.

Result.—The temperature reaction in virus sheep 5 was normal (below 104) up to 2nd February. On the 3rd it rose to 104.4 and 105.6; 4th 105.6 and 107.2; 5th 106.0 and 104.0; 6th 103.8 and 104.4; 7th (a.m.) 105.2. From 8th February onwards the temperature remained normal. This reaction was of medium intensity, lasting 5 days, with a maximum temperature of 107.2°.

(b) EXPERIMENTS WITH *Aedes caballus.*

Aedes caballus was used in six experiments in which a total of 124 specimens were injected into susceptible sheep after intervals of from 18 hours to 16 and 17 days.

The virus sheep Nos. 1, 2, 3 and 6 were used for feeding the mosquitoes on.

The mosquito groups consisted of:

Group 1.—Fed on the afternoon of 19th November, 1931, from virus sheep 1, the second day of fever. Temperature 103.8 and 103.2. Of these mosquitoes, hatched from larvae, 19 specimens engorged. Used for experiments 1 and 2.

Group 2.—Remainder of group 1. Fed on 20th November from same virus sheep on third day of fever. Temperature 104.2. 20 Specimens engorged. Used for experiments 2 and 5.

Group 3.—The unengorged mosquitoes of group 2, fed on same virus sheep between 20th November (afternoon) and the following morning. Third day of fever. Temperature 104.2 and 103.8. 23 Specimens engorged. Used for experiments 2 and 5.

Group 4.—Fed on virus sheep 2 on 25th November (afternoon). First day of fever. Temperature about 105.2. 11 Specimens (caught as adults) engorged. Used for experiment 3.

Group 5.—The same material as above fed on virus sheep 2 during the following night. Second day of fever. Temperature 105.2 and 104.8. 36 Specimens engorged. Used for experiment 3.

Group 7.—Fed during the night of 3rd December, 1931, on virus sheep 3. Second day of fever. Temperature 104.8 and 106.5. 51 Specimens (caught as adults) engorged. Used for experiment 6.

Group 8.—Fed on the same virus sheep during the following night. Third day of fever. Temperature 106.9 and 106.2. 29 Specimens (caught as adults) engorged. Used for experiment 6.

Group 14.—Fed on virus sheep 6 during night of 25th February, 1932. Third day of fever. Temperature 106.4 and 105.4. 75 Specimens (hatched from larvae) engorged. Used for experiment 4.

EXPERIMENT 1 (B.T. 1). 5 *Aedes caballus*.
Injection. Interval 18 hours. Sheep 32331.

On 20th November, 1931, this sheep was injected subcutaneously with an emulsion of five specimens of *A. caballus* (group 1), which had fed the previous afternoon on an infected animal.

Reaction.—On 24th November (4 days p.i.) the sheep showed the first rise of temperature, up to 105°, and on the next day 105.2. On the 26th a slight fall occurred, and on the 27th the temperature registered 103.2 in the morning. The same day it rose to 108° only to commence falling the following day again (106.2 and 105.3), and from 1st December, the temperature regained normal where it remained.

Immunity test.—On 11th December, three weeks after the injection of the mosquitoes, the sheep was tested for immunity by the injection of 1 c.c. B.T.V. subcutaneously. During the following three weeks (the day of injection excluded) the temperature reached 104° for only one afternoon. The super-infection was thus negative and the sheep immune.

Subinoculations.—On 25th November, two sheep, Nos. 31722 and 32346, were each injected subcutaneously with 1 c.c. blood from sheep 32331. The former sheep showed a typical reaction with 107° as maximum, whereas in the latter the reaction was very weak or even doubtful.

The result of the main experiment was therefore *positive*, confirmed by immunity test and subinoculation into other sheep.

EXPERIMENT 2 (B.T. 2). 20 *Aedes caballus*.
Injection. Interval 5 days. Sheep 32335.

Into this sheep were injected subcutaneosuly 20 *A. caballus* which had fed approximately 5 days previously on an infected sheep. Seven specimens of group 1 were injected on 24th November, the same number of group 2 the following day, and on the day after 6 of group 3.

Reaction.—On 2nd December, 8 days after the injection of the first group of mosquitoes a rise of ·temperature occurred, 104.3° being registered, which, however, was not maintained, the temperature regaining normal the following day.

Immunity test.—On 15th December, three weeks after the commencement of this experiment, the immunity of the sheep was tested by the subcutaneous injection of 1 c.c. B.T.V. The temperature rose 9 days later, reaching its maximum, 106°, the same day. It remained above 104° for four days. This reaction may be considered as moderate.

Result.—There may have been a slight immunity developed in this case but the experiment would appear to be *negative*.

EXPERIMENT 3 (B.T. 4). 30 *Aedes caballus*.
Injection. Interval 5 days. Sheep 32303.

Into this sheep 30 specimens of *A. caballus* were injected after the same interval as in the preceding experiment, 8 specimens of group 4 on 30th November and 22 of group 5 the following day.

Reaction.—On 8th December, 7 days after the injection of the last group of mosquitoes, a weak, but quite definite, febrile reaction commenced, which, however, lasted only four days. On 8th December the temperature registered 102.8 and 104.5°; 9th 104 and 105°; 10th 103.3 and 105.9; 11th 103.6 and 104.0. The temperature then returned to normal, remaining thereafter under 103.8°.

Immunity test.—On 23rd December, 22 days after the last injection of mosquitoes, the immunity test consisting of 1 c.c. B.T.V. subcutaneously, was applied. The temperature rose to 104.7 on 28th December and two days later reached 107. The whole reaction lasted 4 days, and was quite typical.

Subinoculation.—Blood from this sheep, taken on 10th December during the short febrile reaction, was injected on that date into sheep 32149. During an observation period of three weeks this sheep never showed a temperature surpassing 104° for longer than half a day. The sheep was tested for immunity three weeks after the injection by the subcutaneous inoculation of B.T.V. A marked febrile reaction resulted, lasting 9 days and showing a maximum of 108°.

The *result* of this experiment must be regarded as *doubtful*, probably as negative. There was a certain febrile reaction, just at the time when it could be expected, but it was not very marked. The immunity test was positive, the sheep therefore not having acquired any immunity. Furthermore, a subinoculation of blood into a susceptible sheep was negative.

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EXPERIMENT 4 (B.T. 14a). 40 *Aedes caballus.*

Injection. Interval 7 days. Sheep 31713.

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This sheep was injected subcutaneously on 4th March, 1932, with 79 mosquitoes belonging to six different species of *Aedes*, viz., 40 *A. caballus*, 3 *A. dentatus*, 2 *A. hirsutus*, 30 *A. lineatopennis*, 1 *A. punctothoracis* and 3 *A. vittatus*. All these mosquitoes had been fed about 7 days previously on an infected sheep. The 40 *A. caballus* which alone concern us here belonged to group 14.

Reaction.—The day following the injection a slight rise in temperature, to 104.2°, took place, but thereafter the temperature remained constant, varying between 101.8 and 103.8°.

Immunity test.—On 5th April, one month after the injection of mosquitos this sheep was injected with 2 c.c. B.T.V. Four days later the temperature rose, passing 104 and reaching 107.4° on the 7th day p.i. The temperature reaction was marked and lasted seven days.

Result.—As shown by the immunity test the sheep had remained fully susceptible and the experiment was thus negative.

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EXPERIMENT 5 (B.T. 5). 14 *Aedes caballus.*

Injection. Interval 15 days. Sheep 32333.

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14 Mosquitoes, which had fed approximately 15 days previously from an infected sheep during the third day of its fever reaction were injected into the above sheep as follows:—9 Specimens of group 2 on 5th December, 1931, and two days later 5 of group 3.

Reaction.—During an observation period of three weeks the animal showed no typical temperature reaction, except that the day following the second injection the temperature rose to 104.2° and later, on the 6th, the 12th and the 15th days after the first injection temperatures of 104.3, 106.4 and 104.2° were recorded. These temperatures were, however, not maintained for periods of longer than ½ day in each case, and were therefore considered as of no consequence.

Immunity test.—On 28th December, three weeks following the last injection of mosquitoes the sheep was injected with 1 c.c. B.T.V. Four days later the temperature reached 105.6°, and after a further period of two days 106.4°, was recorded which constituted the maximum. The reaction persisted for five days and though not marked was quite typical.

Subinoculation.—1 c.c. blood from this sheep taken one day after the short rise of temperature to 106.4°, was injected into sheep 31236 on 18th December. During an observation period of 27 days the temperature did not exceed 103.4° This sheep was then subinoculated with 1 c.c. B.T.V. and seven days

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later the temperature rose to 106.2°. The whole reaction lasted 2-2½ days and was not very marked. There existed therefore the possibility of a certain amount of immunity having been developed.

Result.—In our opinion the experiment must be regarded as *negative*, as the sheep had shown no typical reaction and later proved to be susceptible to bluetongue. Furthermore, a subinoculation of blood into another sheep failed.

EXPERIMENT 6 (B.T. 10). 15 *Aedes caballus*.

Injection. Interval 16-17 *days. Sheep* 32340.

In this experiment 15 *A. caballus*, 9 of group 7 and 6 of group 8, were injected on 26th December, 1931. These mosquitoes had fed 16-17 days previously on an infected animal during the second and third days of fever.

Reactions.—Exacerbations to 104° occurred during the first week following the injection, but thereafter 103.4° was the maximum temperature recorded in the course of the observation period of 25 days.

Immunity test.—On 15th January, 1932, the sheep was inoculated with 1 c.c. B.T.V., the injection being followed by a marked reaction, 106°, constituting the maximum, was reached on the 7th day p.i., and on the 11th day the temperature fell to 102°. The temperature remained above 104° for four days.

Result.—This experiment was therefore *negative*.

(c) EXPERIMENTS WITH *Aedes lineatopennis*.

With *A. lineatopennis* four experiments were conducted and 112 specimens injected into susceptible sheep.

Virus sheep Nos. 1, 4, 5 and 6 were used, and the mosquito groups consisted of:

Group 1.—Fed on virus sheep 1 on 20th November (afternoon). Third day of fever. Temperature 104.2°. Four specimens (caught as adults) engorged. Used for experiment 7.

Group 2.—Fed on the same virus sheep during the following night. Third day of fever. Temperature 104.2 and 103.8°. 26 Specimens (caught as adults) engorged. Used for experiments 7 and 9.

Group 5.—Fed on virus sheep 5 during the night of 4th to 5th February. Second to third day of fever. Temperature 107.2 and 106.0°. 90 Specimens (caught as adults) engorged. Used for experiment 10.

Group 6.—Fed at same time on virus sheep 4. Third day of fever. Temperature 106.2 and 105.3°. 42 Specimens (caught as adults) engorged. Used for experiment 10.

Group 7.—Fed during the following night on same virus sheep. Fourth day of fever. Temperature 104 and 105°. 40 Specimens (caught as adults) engorged. Used for experiment 10.

Group 8.—Fed on virus sheep 5 during the same night. Third to fourth day of fever. Temperature 104 and 103.8°. 32 Specimens (caught as adults) engorged. Used for experiment 10.

Group 9.—Fed on virus sheep 6 during the night 25th to 26th February. Third day of fever. Temperature 106.4 and 105.4°. 42 Specimens (reared from larvae) engorged. Used for experiment 8.

EXPERIMENT 7 (B.T. 3). 20 *Aedes lineatopennis*.
Injection. Interval 5 *days. Sheep* 31848.

20 *A. lineatopennis* were injected into this sheep about five days after their infective meal. They were comprised of 3 of group 1, injected 25th November, 1931, and 17 of group 2 injected the following day. They had fed on an infected sheep during the third day of fever, its temperature of 104.2° at the time, however, having been very low.

Reaction.—During an observation period of three weeks the sheep showed no typical febrile reaction. The temperature passed 104° shortly after the injection and again four days after the second injection, but in each case was maintained for only ½ day.

Immunity test.—On 17th December the sheep was inoculated with 1 c.c. B.T.V. The temperature commenced rising four days later, the fever lasting five days with a maximum of 105.6°. The reaction was thus very mild.

Result.—The sheep being susceptible, the experiment must be regarded as *negative.*

EXPERIMENT 8 (B.T. 14b). 30 *Aedes lineatopennis.*

Injection. Interval 7 days. Sheep 31713.

Into this sheep 30 *A. lineatopennis* of group 9 were injected on 4th March, 1932, together with 49 specimens of five other *Aedes* species.

The course of the experiment has already been described under experiment 4, and there the result was shown to be *negative.*

EXPERIMENT 9 (B.T. 6). 2 *Aedes lineatopennis.*

Injection. Interval 16 days. Sheep 31683.

On 12th December, 1931, two *A. lineatopennis* of group 2 which had had an infective feed 16 days previously were injected into this sheep.

Reaction.—No febrile reaction occurred throughout the observation period of more than five weeks, although 13 days p.i. the temperature exceeded 104° for a period of half a day.

On 14th January, 1932, this sheep was used in experiment 17 (*Aedes vittatus* as a control, but with negative results.

The *immunity test* was conducted on 11th February by the subcutaneous inoculation of 1 c.c. B.T.V. The resultant febrile reaction, with an incubation period of 6 days, a duration of 5 days and with maximum of 106.4°, was typical.

Result.—The experiment must, therefore, be regarded as *negative.*

EXPERIMENT 10 (B.T. 13). 60 *Aedes lineatopennis.*

Injection. Interval 17-19 days. Sheep 31703.

In this experiment 60 *A. lineatopennis* of groups 5 and 8 were injected on 22nd February, 1932. The mosquitoes had fed on the second to fourth days of the fever reaction on virus sheep 5 and 6, i.e. between February 4th p.m. and 6th a.m., thus 17 to 19 days before.

Reaction.—On the 2nd, 3rd and 8th days following the injection temperatures between 104 and 104.4° were recorded, otherwise the temperature remained normal. On 3rd March, the 10th day, p.i. a *definite fever reaction* set in which lasted until 10th March, i.e. 8 days. The maximum was 106.2° and on three days the temperature remained above 105°. Although of medium intensity this reaction was nevertheless quite definite. [For exact temperatures of virus sheep 8 (p. 20).]

Immunity test.—This sheep was tested for immunity on 5th April, 43 days after the injection of mosquitoes, by the subcutaneous injection of 2 c.c. B.T.V. During an observation period of three weeks the temperature remained normal, varying between 101.8 and 103.4°. A *complete immunity* had therefore been acquired.

Subinoculation.—On 10th March, two sheep, 31556 and 32269, were each subinoculated with 2 c.c. blood from the above-mentioned animal, taken the day after the height of the febrile reaction. Both these sheep showed typical bluetongue reactions. In the first case the temperature commenced rising on the fifth day, p.i. and returned to normal on the 13th day, exceeding 104° on seven days with 106° as maximum. The second sheep reacted on the sixth day, the temperature remaining above 104° for six consecutive days with a maximum of 106°. On 10th April, 31 days after the injection of blood from the experimental animal, the immunity of these two sheep was tested by the inoculation of 2 c.c. B.T.V. The temperatures remained normal throughout the observation period of three weeks, varying between 101.4 and 103.4 in the first case and 102.3 and 104° in the latter animal.

Result.—This experiment is therefore definitely *positive.* The injection of the infected mosquitoes was followed, after an incubation period of 10 days, by a typical bluetongue reaction and the sheep later proved to be immune.

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Subinoculations of blood into two susceptible sheep produced typical temperature reactions, these sheep subsequently developing complete immunity to bluetongue.

(d) Experiments with other Aedes Species.

Apart from *A. caballus* and *A. lineatopennis*, small numbers of four other *Aedes* species were used, viz., *A. vittatus*, *A. dentatus*, *A. hirsutus* and *A. punctothoracis*.

Virus sheep Nos. 3 and 6 and mosquito groups:

A. vittatus, group 1.—Fed on virus sheep 3 during the night, 4th to 5th December, 1931. Third day of fever. Temperature 106.9-106.2°. Seven specimens (reared from larvae) engorged. Used for experiment 11.

A. vittatus, group 3.—Fed on virus sheep 6 during the night, 25th to 26th February, 1932. Third day of fever. Temperature 106.4-105.7°. Three specimens (reared from larvae) engorged. Used for experiment 12.

A. dentatus, group 3.—Fed on virus sheep 6 together with *A. vittatus*, group 3. Four specimens (reared from larvae) engorged. Used for experiment 12.

A. hirsutus, group 4.—Fed together with *A. vittatus*, group 3. 3 specimens (reared from larvae) engorged. Used for experiment 12.

A. punctothoracis, group 2.—Fed together with the preceding group.—1 specimen (reared from larvae) engorged. Used for experiment 12.

Experiment 11 (B.T. 7). 6 *Aedes vittatus*.

Injection. Interval 5 days. Sheep 32308.

On 10th December, 1931, 6 *A. vittatus* of group 1 were injected into this sheep. The mosquitoes had fed five days previously on an infected sheep during the third day of fever. Another injection of mosquitoes into his sheep was made seven days later, but this will be referred to under experiment 16 in the following chapter.

Reaction.—The temperature remained normal for 19 days, when a sudden but short rise to 105.6° took place. This was followed by a period of three weeks in which the temperature remained normal. During the following week fluctuations to 104 and 104.8° occurred.

Immunity test.—On 27th January, after an observation period of about seven weeks, the sheep was inoculated with 2 c.c. B.T.V. Seven days later the temperature rose to 105°, the reaction being mild but typical, lasting 4½ days, with 105.8° as maximum.

Result.—This experiment was negative. No typical reaction had occurred following the injection of mosquitoes and the sheep had remained susceptible.

Experiment 12 (B.T. 14c). *Aedes vittatus, A. hirsutus and A. punctothoracis.*

Injection. Interval 7 days. Sheep 31713.

Into this sheep six different species of mosquitoes were injected, which included *A. caballus* and *A. lineatopennis* mentioned under their respective heads, and 3 *A. vittatus*, group 3, 3 *A. dentatus*, group 3, 2 *A. hirsutus*, group 4 and 1 *A. punctothoracis*, group 2.

A full description of this experiment has been given under experiment 4 (*Aedes caballus*).

The result, as mentioned therein, was *negative*.

(e) Results of the Injection of Mosquitoes infected with Bluetongue Vaccine Virus.

In 12 experiments we injected into susceptible sheep 251 mosquitoes which had fed on sheep experimentally infected with a

vaccine strain of bluetongue virus from $\frac{1}{2}$ to 19 days previously. These mosquitoes were of the following species:—

Aedes cabalus, 124 specimens.
Aedes dentatus, 3 specimens.
Aedes hirsutus, 2 specimens.
Aedes lineatopennis, 112 specimens.

Aedes punctothoracis, 1 specimen.
Aedes vittatus, 9 specimens.

In the case of two of these experiments the transmission was successful.

In the first of these two experiments (experiment 1) 5 *Aedes caballus* were injected $\frac{1}{2}$ day after having fed on an infected sheep. The febrile reaction in the experimental animal was very marked, the maximum temperature being as high as 108°.

This result indicated that 5 mosquitoes are capable of taking up sufficient virus to reproduce the disease.

This was certainly remarkable in view of the fact that the reaction in the virus sheep from which the mosquitoes had fed had been very weak. In fact, had such a reaction occurred in our experimental sheep, we would have been inclined to regard it as doubtful or even negative.

The second positive experiment (experiment 10) is of more importance. It served to demonstrate that *the bluetongue virus is capable of persisting in* Aedes lineatopennis *in a fully virulent form for periods of at least 17 days.*

60 Specimens were injected subcutaneously, and after an incubation period of 10 days a typical fever reaction resulted, which lasted for 8 days. When injected almost $1\frac{1}{2}$ months later with the same strain of virus the sheep proved to be completely immune. Two other sheep, subinoculated with blood from this animal taken during its reaction, showed typical reactions, themselves later acquiring complete immunity.

The other experiments were all negative with the exception of one (viz.: experiment 3), which may be looked upon as doubtful. In this case a somewhat atypical reaction occurred which could possibly have been due to a mild infection with bluetongue, but when tested for immunity the sheep proved to be normally susceptible.

The negative experiments and the one doubtful case embody the injection of 186 mosquitoes at varying intervals after the infective meal as follows:—

5 *to* 7 *days after feeding.*
  90 *Aedes caballus.*
  3 *Aedes dentatus.*
  2 *Aedes hirsutus.*
  50 *Aedes lineatopennis.*
  1 *Aedes punctothoracis.*
  9 *Aedes dentatus.*

15 *to* 17 *days after feeding.*
  29 *Aedes caballus.*
  2 *Aedes lineatopennis.*

We will here limit the discussion to the experiments with *Aedes caballus* and *A. lineatopennis* as the number of specimens of the other species used was very small.

We obtained one positive result with 112 *Aedes lineatopennis* injected and only negative results with *A. caballus*, with the exception of experiment 1, when 5 *A. caballus* injected half a day after feeding produced a positive reaction. This case, however, we do not regard as being of much importance in view of the actual transmission as not sufficient time was allowed for the development of the virus in the insect host. The number of experiments conducted is certainly not sufficient to justify the conclusion that one species is a probable transmitter and the other not.

The question presents itself, What can the reason be for the negative results? The chance of the virus developing in the mosquito may be quite small. This may be an important factor. It will be recalled that the virus which we used had been passed through a large number of generations from sheep to sheep by subinoculation in the laboratory. By these successive subinoculations it had been transformed into a vaccine virus, a much attenuated strain, and thus one profound biological alteration had taken place. Another alteration, a change, possibly a reduction, in its developmental capacity in the insect host would, therefore, not be at all surprising.

Another possibility is that the different virus sheep may not have been equally suitable for infecting the mosquitoes. We used 6 sheep in these tests. On virus sheep 4 and 5 the *A. lineatopennis*, which transmitted the infection, were fed. On the others the following mosquitoes fed : —

Sheep 1, 34 *A. caballus*, 22 *A. lineatopennis*. Result negative.
Sheep 2, 30 *A. caballus*, nil. Result doubtful.
Sheep 3, 15 *A. caballus*, nil. Result negative.
Sheep 6, 40 *A. caballus*, 30 *A. lineatopennis*. Result negative.

In virus sheep 1 the reaction was a very mild one, and only exceptionally would we utilize such a case for feeding mosquitoes on. However, on this same sheep those mosquitoes fed which, injected the following day, produced a marked reaction in experiment 1. The other mosquitoes which fed from virus sheep 1 must therefore also have taken up sufficient virus.

Sheep 2 gave the doubtful case.

In the case of sheep 3 only 15 specimens were used.

Sheep 6 is, however, quite remarkable in that 79 mosquitoes comprising 40 *A. caballus* and 30 *A. lineatopennis*, which fed from it, were injected 7 days later without result, notwithstanding the fact that the febrile reaction was very marked.

It is, therefore, not unreasonable to suppose that the virus in one sheep is more capable of developing in mosquitoes than is that in another sheep. On the other hand, as the same virus strain was used to infect all the animals, the varying results experienced may have been due to a general reduction in the developmental capacity of the strain in mosquitoes. Apart from this only a *certain period* in the duration of the febrile reaction may be suitable for the infection of the mosquitos. We were unfortunately not able to carry out a sufficient number of experiments to definitely ascertain this point.

IV. EXPERIMENTS WITH VIRUS COLLECTED FROM A DOUBTFUL CASE OF BLUETONGUE PRODUCED BY THE INJECTION OF MOSQUITOES FED ON B.T.V. VIRUS.

In the preceeding section we discussed (experiment 3) a doubtful case of bluetongue produced by the injection of *A. caballus* 5 days after feeding. The temperature had suggested a mild reaction of bluetongue, and we hoped that this strain, which we considered had displayed some slight aptitude for developing in the insect, would regain its natural virulence in time by further passage through mosquitoes. It must be mentioned that the result of the immunity test did not become known until after the following experiments had been conducted.

In this series of experiments the same procedure was adopted, viz., the subcutaneous injection of emulsified mosquitoes at intervals of 5 to 20 days after the infective meal.

We used 57 specimens of mosquitoes belonging to three species of *Aedes*, viz., *A. dentatus*, *A. hirsutus* and *A. vittatus*, as we had no others at our disposal at that time.

For the feeding of the mosquitoes the following sheep was used :—

Virus sheep 7 (sheep 32303). This sheep was the same as that used in experiment 3 (*Aedes caballus*), and the course of the temperature reaction has been fully described under that experiment.

A. Experiments with *Aedes dentatus*.

Only one experiment, with 9 specimens, was made with this species.

Virus sheep 7.

Mosquito group 2.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of fever. Temperature 105.9-103.6°. 14 specimens (reared from larvae) engorged. Used for experiment 13.

Experiment 13 (B.T. 8). 9 *Aedes dentatus*.
Injection. Interval 5-6 days. Sheep 32046.

On 17th December, 1931, 9 *A. dentatus* of group 2 were injected into the above sheep which, 5-6 days previous, had fed on an infected sheep during the third day of its febrile reaction.

Reaction.—For the first 11 days the temperature remained normal. On 29th December it rose from 103.6° (a.m.) to 105.2°, falling the following morning to 103°, but rising again that afternoon to 106°. Thereafter it remained normal for one week only, to rise again to 105° on 8th January, where it remained for only half a day. This was followed by three days of normal temperature, when a further rise occurred. On 12th January, 104.2 and 105.0° were recorded; on the 13th 104.8 and 104.9°, and on the 14th 104.2 and 102.6°. Thereafter no further abnormal temperatures were registered, although the sheep was kept under observation for the following three months to ascertain whether these sudden temperature elevations were regular or periodic occurrences.

On 21st March the *immunity test* was applied, 2 c.c. B.T.V. being injected. This injection was followed by a reaction six days later, the temperature remaining elevated (above 104°) for 5½ days, with a maximum of 106.8°. The sheep had therefore acquired no immunity.

Subinoculation.—On 31st December, the day following the second rise in temperature, a subinoculation of 1 c.c. blood from this sheep was made into sheep 31979. Four days later a rise occurred, 106° being reached, followed

on the next day by temperatures of 105.2 and 103.4°. It then returned to normal, but on the 13th day, p.i. there was another short rise to 105.2°. The sheep was kept under observation for over 11 weeks, during which period the temperature, although slightly irregular, only three times exceeded 104 for half a day in each case. On 21st March this sheep was inoculated with the same virus as that employed in the case of the original sheep. The ensuing reaction was very marked, persisting for 6½ days with a maximum of 106.8°.

Result.—It is rather difficult to form any definite conclusion in this case and we prefer to regard the result as *doubtful.* The original sheep, which, prior to the mosquito injection had registered a very constant temperature, showed a somewhat doubtful reaction after an incubation period of 13 days. Furthermore, in the sheep subinoculated from it a slight temperature reaction occurred but in neither case was any immunity developed.

B. EXPERIMENTS WITH *Aedes Hirsutus.*

Aedes hirsutus was made use of in two experiments wherein 26 specimens were injected 5 and 16 days after the infective feed.

Virus sheep 7 and mosquito group:

Group 3.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of fever. Temperature 105.9 and 103.6°. 29 specimens (reared from larvae) engorged. Used for experiments 14 and 15.

EXPERIMENT 14 (B.T. 9). 20 *Aedes hirsutus.*
Injection. Interval 5-6 days. Sheep 32118.

On 17th December, 1931, 20 *Aedes hirsutus* of group 3 were injected. These mosquitoes had fed 5-6 days before on an infected sheep during the third day of a febrile reaction.

Reaction.—A rise in temperature occurred on 25th December, the readings being 104.6, and a little later on 105.6, followed the next morning by 103.8°. Unfortunately morning temperatures only were taken (holidays). On 30th December and 13th January temperatures of 106 and 104.8°, respectively, were recorded, which were, however, only fluctuations. Further fluctuations of 106 and 106.4° occurred on 21st and 25th January, followed by normal temperatures for the remainder of the observation period of almost 10 weeks.

Immunity test.—On 5th April, 1932, 110 days after the injection of the mosquitoes, the immunity was tested by the injection of 2 c.c. B.T.V. A marked febrile reaction followed, commencing 6 days later and lasting for 5 days with a maximum temperature of 107°.

A *subinoculation* of 1 c.c. blood from this sheep was made into sheep 31764 on 28th December, 3 days after the slight initial temperature reaction referred to above. From 31st December to 9th January, the 4th to the 12th day following the inoculation, the sheep showed an extremely irregular temperature exceeding 104° on 5 days (on 2 days afternoon temperatures were not recorded), whereon 106°, 106.7°, 105.2°, 104.8° and 105° were registered. These elevations were in each case followed on that or the following day by drops in temperature to 103° or lower. The temperature remained more regular during the ensuing four weeks exceeding 104° for half a day on only one occasion. On 10th February, the immunity test was applied, 1 c.c. B.T.V. being injected. After an incubation period of 6 days a short but definite reaction occurred which lasted for 2¼ days, 106.8° being the maximum temperature.

The results of this experiment are not altogether clear. A slight temperature reaction followed the injection of mosquitoes, but the immunity test clearly demonstrated that no immunity had been acquired. Another sheep, sub-inoculated with blood from this animal, showed a very irregular temperature over a period of 8 days, running up as high as 106.7° on one occasion. This reaction was not typical for bluetongue, but similar reactions are met with from time to time in subinoculations of the vaccine virus strain. The reaction following the immunity test of the second sheep was mild, lasting for only 2¼ days, seeming to indicate that some slight immunity had been developed.

The transmission in this experiment must, therefore, be regarded as *doubtful,* in all probability negative.

EXPERIMENT 15 (B.T. 11). 6 *Aedes hirsutus.*

Injection. Interval 16 days. Sheep 32298.

This sheep was injected with 6 *Aedes hirsutus* of the same group as those used in the preceding experiment, but 16 days after the infective meal.

Reaction.—The temperature remained normal for 12 days, varying between 101.5 and 104°. On 10th January, a mild temperature reaction commenced, lasting three days, and accompanied by discharges from the nostrils. Temperatures of 106 and 103.2° were recorded on 10th January, on the 11th 105.2 and 105°, on the 12th 103.4 and 105.2° and on the 13th 103.2 and 102.6°. During this reaction an occasional rise in temperature up to 106.2° was noted. The animal was kept under observation for the following 11 weeks, during which time it showed a very regular temperature, only twice, for periods of half a day at a time, reaching 104°.

Immunity test.—On 5th April, 99 days after the injection of mosquitoes the immunity was tested by the injection of 2 c.c. B.T.V. Six days later a very marked febrile reaction followed, lasting 7 days, with a maximum temperature of 108°.

Subinoculation.—Blood from this sheep taken on 11th and 14th January, during and shortly after the reaction referred to above, was injected into sheep 32349 on the latter date. Throughout the course of an observation period of more than 12 weeks no definite febrile reaction occurred. On the 4th, 6th and 7th days, p.i. slight rises in temperature to 104 and 104.4° occurred, and on the 20th day, p.i. 105° was exceeded for half a day, otherwise it remained normal. 88 days after the injection (i.e. on 11th April) the sheep was tested for immunity by the subcutaneous injection of 1 c.c. B.T.V. The reaction which followed was very marked lasting 8 days with a maximum temperature of 106.7°.

On 27th April a further sheep (No. 34738) was injected with preserved blood taken on 11th January from the original sheep (No. 32298), but in this case 10 c.c. were injected intrajugularly in order to increase the chances of infection. No definite reaction, however, occurred. Temperatures of 104° were exceeded several times for not longer than half a day on each occasion, but this had also been the case before the sheep was infected. On 28th May the animal was tested for immunity by the injection of 1 c.c. B.T.V. A typical reaction lasting 4 days with temperatures up to 106.4° followed after an incubation period of 4 days.

Result.—This experiment has to be regarded as *negative.* A mild temperature reaction was shown, but when tested for immunity the sheep proved to be normally susceptible and also subinoculations of blood, taken during the febrile reaction, into 2 susceptible sheep failed.

C. EXPERIMENTS WITH *Aedes Vittatus.*

With this species two experiments were conducted wherein 22 specimens were injected 5 and 20 days after feeding on an infected sheep.

Virus sheep 7 and mosquito group:

Group 2.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of reaction. Temperature 105.9° and 103.6°. 40 specimens (reared from larvae) engorged. Used for experiments 16 and 17.

EXPERIMENT 16 (B.T. 7). 14 *Aedes vittatus.*

Injection. Interval 5 days. Sheep 32308.

This sheep had been used once before, viz., in experiment 11, where it had been injected with 6 *A. vittatus* group 1.

On 17th December it was injected with 14 *A. vittatus* of group 2 which had fed 5-6 days before on sheep 7 during the third day of its febrile reaction.

Reaction.—No typical reaction resulted. Once only, 13 days after the injection of mosquitoes, the temperature exceeded 105° for half a day.

Immunity test.—On 27th January after an observation period of 20 days the sheep was injected with 2 c.c. B.T.V. The resultant reaction was quite typical, lasting 4-5 days, with a maximum temperature of 105.8°.

The *result* of this experiment was definitely *negative.*

EXPERIMENT 17 (B.T. 12). 8 *Aedes vittatus.*

Injection. Interval 20 days. Sheep 32288.

For this experiment 8 *Aedes vittatus* of the same group as in the preceding experiment were used on 31st December, 1931, but the injection was made 20 days after their initial feeding.

Reaction.—Four days following the injection of the mosquitoes (i.e. on 4th January) the temperature rose rapidly to 106°, where it remained throughout the day. On the 5th 105.8 and 107° were recorded, on the 6th 106.7 and 105°, the 7th 104 and 103.7°, and on the 8th 104.4 and 104°. It then returned to normal and remained below 104° for the following 18 days. This temperature reaction was certainly very marked, and it closely resembled the usual bluetongue reactions. However, the incubation period of four days was very short, especially when we take into consideration the fact that only a very small quantity of virus could have been injected with the crushed mosquitoes. Following upon the temperature reaction slight clinical symptoms were manifested consisting of distinct warmth of the feet and coronets, slight discharges from both nostrils and frequent licking of the lips.

Immunity test.—On 27th January the sheep was tested for immunity by the injection of 2 c.c. B.T.V. subcutaneously. Seven days later the temperature rose to 105.4°, remaining at 105 during the following day, but falling the day after below 104°. Two fluctuations to 105° occurred on the 12th and 20th day, p.i. but they lasted only half a day each. The temperature reaction following upon the immunity test injection was very weak, lasting only 1½ days, with a maximum of not even 106°.

Subinoculation.—On 7th January a sheep, 31696, was subinoculated subcutaneously with 1 c.c. blood from the preceding sheep taken two days previously. No typical febrile reaction followed this injection. Twice only, viz., 4 and 11 days p.i., short rises of temperature to 106 and 105.2° respectively, occurred. Five weeks after the subinoculation of blood, i.e. on 10th February, 1 c.c. B.T.V. was injected. This was followed by a marked temperature reaction lasting 8-9 days with a maximum of 106.6°.

Another sheep, 32299, was subinoculated with 1 c.c. of the same material as sheep 31696, on 14th January. No temperature reaction resulted with the exception of a few slight fluctuations up to 104°. On 11th April, twelve weeks after this subinoculation, 1 c.c. B.T.V. was injected, which resulted in a pronounced temperature reaction lasting 4 days with 108.8° as maximum.

Together with the above sheep a third, viz. 31683, was subinoculated with the same material. This sheep had been used in a negative experiment (see experiment 9, *A. lineatopennis*). Again no typical reaction ensued. 16 days, p.i. a short rise in temperature to 105° took place. On 10th February this sheep was inoculated with 1 c.c. B.T.V., which was followed by a typical reaction of 5½ days' duration, showing a maximum temperature of 106.4°.

On 27th April we injected yet another sheep, No. 34690, with the same preserved material as in the preceding cases. In this case, however, 10 c.c. was injected intrajugularly. For 21 days the temperature remained normal (not exceeding 104°). Then a mild reaction followed, the 19th 105.6, the 20th 103.8 and 104.8, and the 21st 103.4 and 104.8 being recorded, whereafter the temperature dropped to normal again. On 28th May the sheep was tested for immunity by injection of 1 c.c. B.T.V. After an incubation period of 7 days a clear reaction, lasting 6 days, with 107.4 as a maximum, followed.

The *result* of this experiment has to be regarded as *doubtful*. Four days after the injection of the mosquitoes a distinct temperature reaction lasting 3 days with 107° as maximum of temperature appeared, followed by slight clinical symptoms. Except for the short incubation period, the reaction resembled a typical bluetongue reaction. Besides, the temperature reaction following upon the immunity test was very weak, lasting only 1½ days. Blood taken during the end of the febrile reaction failed, however, to transmit the disease to 4 susceptible sheep.

D. Results of the Injection of Mosquitoes Fed on a Doubtful Case produced by B.T.V. which had been subjected to Passage through Mosquitoes.

On a sheep showing a mild bluetongue-like reaction which had been produced by the injection of a number of *A. caballus* fed five days previously on a true experimental case of bluetongue (B.T.V.). we fed a number of *A. dentatus*, *A. hirsutus* and *A. vittatus*. These were injected into susceptible sheep at intervals of from 5 to 20 days following the feed.

The results of these experiments were as follows:—

Injection of 9 *A. dentatus* 5-6 days after feeding (experiment 13) was followed 13 days later by a temperature reaction lasting 2 days with 106° as maximum. A subinoculation of blood into another sheep produced a similar short reaction four days after the injection. Both these sheep when tested for immunity about three months later proved to be susceptible. The result was looked upon as doubtful.

20 *Aedes hirsutus* (experiment 14) were injected after the same interval, resulting in a short febrile reaction 8 days later in which the temperature exceeded 105°. This sheep was found to be still normally susceptible almost 4 months later. A sheep subinoculated with 1 c.c. blood from this case showed an extremely irregular temperature, which rose as high as 106·7°, from the 4th to the 12th day following injection. The immunity test, conducted one month later, was followed by a very mild reaction of only 2½ days' duration. This case must also be regarded as somewhat doubtful.

Another lot of *A. hirsutus*, consisting of 6 specimens, was injected after a period of 16 days (experiment 15). 12 Days later a temperature reaction commenced closely resembling a mild bluetongue reaction, 106° being the maximum temperature recorded. Three months later, however, this sheep was found to be normally susceptible. A sheep, injected with 1 c.c. blood taken during the course of this reaction failed to react, although it was fully susceptible as demonstrated at a later date. A further sheep was injected later on with 10 c.c. blood given intrajugularly and this also failed to show any typical fever reaction. This experiment also appears to be doubtful.

A further experiment (experiment 16) wherein 14 *Aedes vittatus* were injected five days after feeding, gave negative results.

8 *A. vittatus* of the same group were injected into another sheep after a longer interval, viz., 20 days (experiment 17). A very distinct temperature reaction combined with slight clinical symptoms made its appearance 4 days later, lasting 4-5 days and showing a maximum temperature of 107°. This reaction very closely resembled that of a typical bluetongue reaction. The immunity test injection produced a very weak reaction persisting for only 2 days, the maximum temperature not exceeding 105.4°. An immunity appears to have been developed, not sufficient, however, to completely prevent a reaction, but nevertheless sufficiently strong to considerably modify it. Three sheep were injected with 1 c.c. blood each, from this sheep taken during the febrile reaction. None of these sheep reacted typically

and all three were later proved to be susceptible to injection of known virulent blood. Furthermore, a fourth sheep was injected with 10 c.c. blood intrajugularly and still no typical temperature reaction ensued. From the results obtained by the injection of mosquitoes in the first instance, we must regard this experiment as doubtful.

The results obtained in these experiments are certainly of interest. We started off with a sheep showing a mild temperature reaction not followed by immunity, and in most of the subsequent cases, produced by injections of mosquitoes fed from this sheep, marked reactions resulted, some of which were indistinguishable from true vaccine strain bluetongue reactions. These, however, were not followed by immuity or at any rate the immuity conferred was extremely weak. Subinoculations of blood from these cases into susceptible sheep did not succeed at all, or, where slight febrile reactions resulted, no immunity was acquired.

The temperature reactions noted may not of course have been connected with bluetongue in any way and may have been purely accidental or coincidental, but in many cases where the sheep were kept under observation for several months the temperatures remained remarkably constant. Furthermore, in most cases the reactions commenced within the limits of the known bluetongue incubation period; in other words, just when they were to be expected. In this connection one must not lose sight of the fact, that immunity does not invariably follow bluetongue, there being notable exceptions recorded, and moreover, that subinoculations with normally virulent virus are not successful in producing results in all cases.

We have regarded these experiments as negative or at most doubtful, which appears to be the only rational conclusion. Had we regarded them as positive, A. hirsutus and A. vittatus would naturally be incriminated as transmitters, which, with the results so far obtained, cannot be assumed.

V. EXPERIMENTS WITH VACCINE VIRUS AFTER ONE DEFINITE PASSAGE THROUGH MOSQUITOES.

In the first section we described an experiment (experiment 10) in which a true case of bluetongue was obtained by the injection of a large number of *Aedes lineatopennis* 17 to 19 days after the infective meal. This case was verified by subinoculations of blood from it into two susceptible sheep and subsequent immunity tests. This appeared to be an excellent case wherewith to carry on the experiments and endeavour to obtain results in the more natural manner, viz., by the actual feeding of mosquitoes.

At that time, about the middle of March, large numbers of *A. caballus* and a fair number of *A. lineatopennis* were available from one or other of our experimental breeding places. *A. hirsutus* was not so plentiful, but could periodically be obtained in moderate numbers. These three species were therefore used in this series of experiments.

It was our intention to commence feeding the mosquitoes on susceptible sheep 14 to 15 days after the infective meal and once again

5 days later, if any specimens still survived. In the event of no typical reactions resulting the remaining mosquitoes would be injected.

In all, 9 experiments were conducted in which 93 mosquitoes fed on susceptible sheep after intervals of 14 to 20 days and 11 specimens were injected 22 to 36 days following the original feed on the reacting sheep.

A. Virus Sheep.

The mosquitoes were fed on the following three sheep:

Virus sheep 8 = sheep 31703 from experiment 10 (into which A. lineatopennis were injected 17 to 18 days after feeding).

Result.—Temperature reaction: 3rd March, 103.2 and 105.8°; 4th 103.6 and 104.0°; 5th 103.4 and 104.2°; 6th (a.m.) 104°; 7th 104.9 and 104.6°; 8th 104.6 and 105.9°; 9th 106.2 and 105.2; 10th 104.5 and 104.6° and 11th March 103.2 and 103.5°. It is rather difficult to estimate in this case where the actual bluetongue reaction commenced, 3rd or 7th March may either be looked upon as the commencement.

Virus sheep 9 = sheep 31556 from experiment 10 (injected on 10th March with blood from virus sheep 8).

Result.—The temperature remained normal until 14th March. On 15th 103 and 104.6°, 16th 103.6 and 103°; 17th 104.8 and 106°; 18th 105.3 and 105°, 19th 105 and 103.8°; 20th (a.m.) 103.8°; 21st 104.3 and 105.1°; 22nd 104.2 and 104.2°; and 23rd 103.3 and 104.1°. From the 24th onwards the temperature remained normal. An injection of B.T.V. on 10th April revealed the presence of complete immunity. In this case 15th March or perhaps 17th March may be regarded as the commencement of the reaction.

Virus sheep 10 = sheep 32269 from experiment 10 (injected together with preceding sheep with blood from virus sheep 8).

Result.—Temperature normal until 14th March. On 15th 102.8 and 104°; 16th 103.2 and 105°; 17th 105.6 and 106°; 18th 105.6 and 105.1°; 19th 104.2 and 105.1°; 20th (a.m.) 104° and on 21st 105.8 and 105°. From the 22nd onwards the temperature remained normal except for two short elevations on the 24th and from the 26th to the 27th. When tested on 10th April this sheep showed complete immunity. The commencement of the reaction may, in this case, be regarded as 15th or 16th March.

B. Experiments with *Aedes caballus*.

A large number of *A. caballus* were fed on the three virus sheep, of which 36 engorged themselves from the original sheep and 410 from the two sub-inoculated cases. This number was very considerably reduced within the first week after feeding, the high mortality resulting from the deposition of large numbers of eggs.

In two experiments we succeeded in feeding 41 specimens on susceptible sheep after an interval of 14 to 15 days, 10 specimens in one experiment after 18-19 days, and 1 specimen remained for injection after an interval of over one month.

Experiment 18 was carried out with the mosquitoes fed on the original case, and experiments 19 to 21 with those infected on the two subinoculated sheep.

Virus sheep 8 to 10 and mosquito groups:

Group 16.—Fed on virus sheep 8 during the night of 9th to 10th March. Seventh (or third) day of fever. Temperature 105.2 and 104.5°. 36 specimens (caught as adults) engorged. Used for experiment 18.

Group 17.—Fed on virus sheep 10 during the night of 17th to 18th March. Third (or second) day of fever. Temperature 106 and 105.6°. 112 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

Group 18.—Fed at the same time as group 17 on virus sheep 9. Third (or first to second) day of fever. Temperature 106 and 105.3°. 186 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

Group 20.—Fed on virus sheep 10 on night of 18th to 19th March. Fourth (or third) day of fever. Temperature 105.1 and 104.2°. 112 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

EXPERIMENT 18 (B.T. 15). 16 *Aedes caballus.*
Feeding. Interval 14-15 *days. Sheep* 32326.

On the night of 23rd to 24th March *A. caballus* group 16 was allowed to feed on this sheep and 12 engorged specimens were obtained. On the 24th the remainder were again placed on this sheep, of which 4 engorged themselves, making a total of 16 engorged specimens. These mosquitoes had fed 14 to 15 days previously on sheep 31703 from experiment 10 during the seventh or third day of the reaction (the actual commencement of the reaction is difficult to ascertain), this sheep having been injected with *A. lineatopennis.*

Reaction.—No typical febrile reaction followed the feeding of the mosquitoes, the temperature remaining normal except for 26th and 27th March, and 9th and 18th April, when it rose to 104°, and on one occasion to 105.2°.

On 19th April the *immunity test* was applied, 1 c.c. B.T.V. being injected. A marked reaction followed on the 4th day, which lasted 6 days and showed a maximum of 107.1°.

Result.—This experiment was therefore *negative* as no typical reaction followed the feeding of the mosquitoes, whereas the sheep proved to be normally susceptible.

EXPERIMENT 19 (B.T. 18). 25 *Aedes caballus.*
Feeding. Interval 14-15 *days. Sheep* 33528.

On 1st April a cage containing *A. caballus*, groups 17, 18 and 20 combined, was placed on the above sheep and during the night 25 specimens engorged themselves. These mosquitoes had fed 14-15 days previously between March 17th (p.m.), and the 19th (a.m.) on virus sheep 9 and 10.

Reaction.—On 7th April, 6 days after the feeding of the mosquitoes, the temperature rose to 106.2°, remained at 105.4 to 106.2° during the following day, and was still 106° the next morning. It then dropped back to normal, where it remained for 9 days. On 19th April a second rise took place, 105.8 and 103.2° being recorded on that day and 106 and 105° on the 20th with 103.6 and 104.8° on the day following. This was followed up to 3rd May by another period of normal temperatures, when 106.5 and 105.5° were registered. These temperatures were maintained, however, for only the one day. During the next 5 days the temperature was again normal.

Subinoculations.—On 8th April, during the first rise of temperature, sheep 34660 was subinoculated with 2 c.c. blood and on 21st April during the second temperature rise, once more with 2 c.c. blood taken on that date. The sheep was kept under observation up to 8th May, 31 days after the first and 18 days after the second inoculation. A temperature of 104° was only surpassed on the day of the first injection, on the 2 following days and on the 8th day, 104.7° being the highest temperature registered. Apart from this the temperature remained normal. On 9th May the immunity of the sheep was tested by the injection of 1 c.c. B.T.V. On 15th May, 6 days later, the temperature began to rise to 105.6°. It then dropped and 4 days later was normal again. The result of this subinoculation therefore was negative, no reaction occurring, the animal being susceptible.

With the same material as that used in the preceding case another animal, sheep 34663, was injected on 8th and 21st April. This sheep was kept under observation up to 12th May, 34 days after the first and 21 after the second inoculation. Only once, 7 days after the first injection, was a temperature higher than 104 (104.2°) registered for ¼ day, otherwise the temperature was normal throughout the observation period. On 12th May 1 c.c. B.T.V. was injected in order to test the immunity. After an unmaintained rise of temperature (104.6°) on 17th May, an abrupt rise up to 107° occurred on 19th May. The temperature remained at 107° for another ½ day, then for 3 days at 105° and from 25th May onwards was normal again. This sheep was, therefore, fully susceptible, and no reaction having followed the subinoculations, this experiment must also be regarded as negative.

Immunity test.—On 9th May, 38 days after the feeding of the mosquitoes, and 29 days after the first temperature reaction, the original sheep of the main experiment was tested for immunity by injection of 1 c.c. B.T.V. On 15th May the temperature reached 104.2°, the next day 107°, but fell one day later back to normal (103°). Then another reaction, lasting about 5 days with 105° as maximum occurred.

Result.—The result of this experiment is somewhat difficult to interpret. Five days after the feeding of the mosquitoes a short but clear reaction (106°) was noticed, certainly occurring earlier than a mosquito-transmitted reaction would be expected. Ten days later another reaction appeared during which temperatures above 104 and 106° as maximum were noticed on 5 days. Subinoculations of blood taken during these exacerbations failed to produce a reaction in susceptible sheep. Furthermore, after the immunity test the sheep reacted, although not absolutely typical, at any rate clearly enough. The reaction after the feeding of the mosquitoes and that which followed the immunity test were of equal intensity. The experiment cannot be regarded as positive nor with certainty as negative, and we prefer therefore to regard it as *doubtful*.

EXPERIMENT 20 (B.T. 21). *Aedes caballus.*
Feeding. Interval 18-19 days. Sheep 33544.

On 5th April the remainder of the mosquitoes used in the preceding experiment, viz. groups 17, 18 and 20 were fed on sheep 33544 and 10 specimens engorged themselves during the night. In this case 18 to 19 days had elapsed since the inital feed on the virus sheep 9 and 10.

Reaction.—The temperature of this sheep remained normal for 11 days. On 18th April it rose from 102.6° to 105°, remained at 104.6° and 105.4° during the following day and fell back from 105 to 103.6° on the third day. Thereafter it remained normal except for a short rise to 104.8° on 25th April.

Subinoculations.—2 c.c. blood were injected into sheep 31841 on 20th April, the last day of the reaction. The sheep was kept under observation for 22 days. No reaction was noticed, the temperature remaining between 101.8 and 103.8°. On 12th May this sheep was tested for immunity by the injection of 1 c.c. B.T.V. Seven days later the temperature began to rise and reached 108.2° on 20th May. The reaction was very marked, the temperature remaining above 105° for 6 consecutive days. As no reaction had followed the original injection the subinoculation was negative.

Together with this animal sheep 34422 was injected with the same material. On the 2nd and 3rd day after the inoculation the temperature was somewhat elevated (104 and 105°), but thereafter normal up to 12th May, when the sheep was tested for immunity by injection of 1 c.c. B.T.V. Ten days later the sheep reacted, the fever lasting 6-7 days, and 107° being the highest temperature noticed. This subinoculation was also negative.

Immunity test.—On 9th May, 34 days after the feeding of the mosquitoes and 19 days after the fever reaction the immunity of sheep 33544 of the main experiment was tested by the injection of 1 c.c. B.T.V. Six days later the temperature reached 105° and on the next day rose further up to 106.8°. It remained in the vicinity of 106 for one more day, commenced falling the next day, at first slightly, and regained normal on 22nd May.

Result.—This experiment is still more difficult to interpret than the preceding. Eight days after the feeding of the mosquitoes a fever reaction commenced, showing its maximum 3 days later. It was not very severe, but was quite typical, occurring in a sheep with normally regular temperatures and at the time when a reaction from the feeding of the mosquitoes could be expected. A subinoculation of the blood, taken during the fever reaction, into two susceptible sheep failed to produce any result and furthermore, the immunity test of the original sheep was quite marked. We therefore prefer to regard this experiment as *doubtful* as well.

EXPERIMENT 21 (B.T. 24). *Aedes caballus.*
Injection. Interval 35-36 days. Sheep 34615.

Of the combined mosquito groups 17, 18 and 20, which had been used in experiments 19 and 20, only one specimen remained one month after the initial feed. We could not induce this specimen to feed again on another sheep, and

on 22nd April it was, therefore, injected after an interval of 35 to 36 days into sheep 34615.

Reaction.—The sheep was kept under observation for 20 days. During this period no reaction occurred, 103.4° being the highest temperature registered.

The *immunity test* was applied on 12th May through injection of 1 c.c. B.T.V. Five days later a temperature reaction began to appear which, however, was not typical and not very marked. It lasted about 6 days with temperatures varying between 103 and 105.2°.

Result.—We regard this experiment also as *negative*, as no reaction followed the injection of the mosquito. The fever following the subsequent virus injection was not marked and perhaps the sheep was slightly immune. We do not think, however, that through this the result of our experiment was largely influenced.

C. Experiments with *Aedes lineatopennis*.

On virus sheep No. 8, i.e. the original case, 44 *A. lineatopennis* were fed, and 92 specimens on the two subinoculated animals together. These constituted practically the end of our supply of this species as the season was almost over and *A. lineatopennis* was becoming comparatively rare. The mortality amongst this species, although considerable, was noticeably less than amongst *A. caballus* whereas they were both kept under identical conditions.

In three experiments we succeeded in feeding 39 specimens, 15 after an interval of 14 to 15 days, 8 after 14 to 20 days and 16 after 18 to 26 days. In a fourth experiment 10 specimens were injected after 22 days.

Experiments 22 and 23 were carried out with the mosquitoes fed on the original case; experiments 24 and 25 with those fed on the subinoculated sheep.

Virus sheep: 8 to 10 and mosquito groups:

Group 11.—Fed on virus sheep 8 during the night of 9th to 10th March. Second (or third) day of fever. Temperature 105.2 and 104.5°. 44 specimens (caught as adults) engorged. Used for experiments 22 and 23.

Group 12.—Fed on virus sheep 10 during the night of 17th to 18th March, Third (or second) day of fever. Temperature 106 and 105.6°. 47 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 13.—Fed at the same time as group 12 on virus sheep 9. Third (or first to second) day of fever. Temperature 106 and 105.3°. 11 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 14.—Fed on the same virus sheep the following night. Fourth (or second to third) day of fever. Temperature 105 and 105°. 15 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 15.—Fed during the same night on virus sheep 10. Fourth or third day of fever. Temperature 105.1 and 104.2°. 19 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Experiment 22 (B.T. 16). 8 *Aedes lineatopennis*.
Feeding. Interval 14-20 *days. Sheep* 31694.

On 23rd March, *A. lineatopennis* (group 11) were placed on this sheep, two specimens feeding during the night. The following day the remainder were put on to this sheep, four more engorged specimens being obtained. On 29th March all the remaining mosquitoes of this group were again allowed to feed on this sheep, and during the night 2 out of the 12 engorged. Mosquitoes were therefore fed on this sheep 8 times, viz., 2 specimens after an interval of 14 days, 4 after 15 days and 2 specimens after 20 days. The mosquitoes had engorged themselves on a sheep during the second and third days of a febrile reaction.

Reaction.—Sheep 31694 was kept under observation for 28 days (21 days after the last mosquitoes had fed) during which period the temperature remained normal, the highest point reached being 103.4°.

Immunity test.—On 20th April the immunity was tested by the injection of 1 c.c. B.T.V. On the 6th day p.i. the temperature rose to 105, fell to 103.1° that afternoon, registered 102.6° and 107° the following day, then 105.2 and 106.4° and on the 9th day p.i. 103.8 and 104.9°. From the 10th day onwards it remained normal. Although a maximum temperature of 107° was recorded the reaction was not very marked, being of a remittant nature and remaining above 104° for a period of less than 48 hours.

Result.—Notwithstanding the somewhat atypical nature of the immunity test this experiment must be regarded as *negative.*

EXPERIMENT 23 (B.T. 17). 10 *Aedes lineatopennis.*
Injection. Interval 22 days. Sheep 31777.

On 31st March, 10 specimens (the remainder of *A. lineatopennis* group 11), which had been used in the preceding experiment, were injected into sheep 31777. The interval between the feeding of the mosquitoes on the virus sheep and their injection was 22 days.

Reaction.—Except for a slight rise the day after injection, the temperature of this sheep remained normal for 12 days. A very slight temperature reaction then commenced which lasted for two days, the temperature recorded on the afternoon of the 13th day being 102.4 and 104.6° and the following morning 103.4 and 104.5°. Throughout the following week the temperature, although fluctuating remained normal.

Immunity test.—On 21st April, 3 weeks after the injection of mosquitoes, the immunity was tested, 1 c.c. B.T.V. being injected. The reaction that followed was not typical. Seven days, p.i. the temperature rose to 105.6°, remained at 104.4 for 48 hours and thereafter returned to normal for 2 days, when another rise up to 104.8° lasting ½ day, followed. During the following week the temperature was again normal.

Result.—It is not possible to express a definite opinion as to the result of this experiment and we have to regard it as *doubtful.* The injection of the mosquitoes was not followed by a definite fever reaction, but on the other hand no typical reaction was obtained by the injection of true bluetongue virus, the same batch of virus which gave a very marked reaction in a large number of other sheep. The sheep may possibly have acquired an immunity prior to the commencement of this experiment.

EXPERIMENT 24 (B.T. 19). 15 *Aedes lineatopennis.*
Feeding. Interval 14-15 days. Sheep 32076.

On 1st April, 1932, the combined groups 12 to 15 of *A. lineatopennis* were place on sheep 32076, and during the night 15 out of the 21 specimens fed. These mosquitoes had fed between 17th and 19th March on virus sheep 9 and 10.

Result.—After an incubation period of 9 days the temperature rose to 104°. Thereafter, one day of normal temperature followed, and on 13th April 102.8 and 104.2° were recorded; the 14th 105.9 and 104.8° and on the 15th 104 and 103.4°. This concluded the fever reaction, but the temperature thereafter remained somewhat higher than during the incubation period, often reaching or surpassing 104°.

Subinoculation.—On 14th April, i.e. the highest point of the fever reaction, two sheep, 34484 and 34531, were injected subcutaneously with 2 c.c. blood each, taken on this day.

The first sheep did not show a typical reaction. In the course of an observation period of 25 days, the highest temperature recorded was 104.6°, 104° being reached or surpassed on the 2nd, 6th to 8th and 14th to 16th days. On the 15th to 16th day the temperature varied between 104.2 and 104.6°, but this was probably also not of the nature of a true reaction. The sheep was tested for immunity by injection of 1 c.c. B.T.V. on 9th May. Seven days later the temperature reached 106.2°, but fell the next day to 103.6°. The following morning another rise made its appearance, the temperature remaining at 106° for 24 hours and then falling back to normal. This subinoculation was therefore negative.

The second sheep, however, showed a very marked reaction after an incubation period of 5 days, which lasted for 7 days, the maximum temperature

attained being 106.6°. For 4 consecutive days the temperature varied between 104.8 and 106.6°, during which time it fell below 105° for only half a day (for full references of the temperature see under virus sheep 13). Slight but typical clinical symptoms were shown, discharges from the nostrils, slight stomatitis and warm feet being evident. Dullness with a general sick appearance was apparent for a number of days. From 26th April onwards the temperature was normal. On 12th May, 28 days after the injection of blood and 17 days after the end of the febrile reaction the sheep was tested for immunity by injection of 1 c.c. B.T.V. The sheep was kept under observation up to 7th June. No reaction followed this subinoculation, 104° being the highest temperature noticed. This subinoculation was therefore positive.

Immunity test.—On 3rd May, 32 days after feeding the mosquitoes and 18 days after the fever reaction, the immunity of this sheep was tested, 1 c.c. B.T.V. being injected. On 9th May the 6th day, p.i., the temperature rose up to 106.6°. The following two days the temperature remained above 106°, 107.6° being registered as maximum. On the third day (12th May) the temperature was still at 106° and the sheep was then killed for other experiments. The course of the fever reaction indicated that the sheep was normally susceptible to bluetongue.

The result of this experiment is somewhat remarkable. Twelve days after the feeding of the mosquitoes a slight fever reaction with a maximum temperature of 105.9° occurred. It may have been a slight attack of bluetongue fever and therefore 2 other animals were subinoculated with the blood. The original sheep was tested for immunity 18 days after this fever reaction and proved to be normally susceptible. The first subinoculated sheep did not show any reaction of importance and later also proved to be susceptible. In the second sheep, however, the inoculation was followed by a typical and marked reaction with slight, but typical clinical symptoms, and when tested for immunity 17 days after the reaction it proved to be totally immune. We maintain that this experiment must be regarded as *positive*, on account of the definite result in one of the subinoculation experiments, notwithstanding the fact that the sheep had not acquired any immunity. The fever reaction following the feeding of the mosquitoes was short and not very marked, and although being in all probability of a true bluetongue nature, as one of the subinoculations proved clearly, it was not strong enough to give the sheep an immunity sufficient to resist a subsequent inoculation of bluetongue virus.

EXPERIMENT 25 (B.T. 22). 16 *Aedes lineatopennis*.
Feeding. Interval 18-26 days. Sheep 33554.

On 5th April, 1932, the remainder of the *A. lineatopennis* (combined) groups 12-15, used in the preceding experiment, were allowed to feed on sheep 33554, and 14 specimens (out of 22) engorged themselves during the night. Seven days later the survivors of this group were given another opportunity of feeding during the night of 12th to 13th April and 2 out of 6 specimens engorged. These mosquitoes had fed originally on virus sheep 9-10, the interval in the case of the 14 specimens having been 18-19 days and in the remaining two 25 to 26 days.

Reaction.—The temperature of the sheep remained normal for the first 8 days, disregarding the second feeding of the mosquitoes. Then on 14th April it rose from 103.4 to 104.8°, the next day registering 104.2°. One day of normal temperature followed, after which a more marked reaction set in. On 17th April, 12 days after the first feeding of the mosquitoes, the temperature remained at 106° throughout the day. The following day 106° and 105.6° were recorded and on the third day of the reaction 104.2 and 104.6°. For two more days a slightly elevated temperature was noted (maximum 103.9°) and thereafter it returned to normal and showed a regular course, remaining for 17 days below 104° except for one sudden rise up to 105°, lasting, however, only ½ day.

Subinoculation.—On 18th April, the second day of the fever reaction, 2 c.c. blood taken on this day were injected into sheep 32302, which was kept under observation for 21 days. During this period only once, 9 days after the injection was a temperature above 104 (104.4°) registered for ½ day. On 9th May 1 c.c. B.T.V. was injected for testing the immunity. On 16th May, 7 days later, the temperature reached 107.1° and 107.6°. It began to drop the next day, but an elevated temperature was maintained for the following 6-7 days. The sheep was fully susceptible and the subinoculation negative.

Together with the preceding animal another sheep, 32317, was injected with the same material. It was also kept under observation for 21 days. Except for some fluctuations, up to 107° on 21st April, to 104.5° on the 25th and to 105° on the 27th, which however were maintained in each case for only ½ day, the temperature remained normal. On 9th May the sheep was tested for immunity by the injection of 1 c.c. B.T.V. This injection was also followed by a marked reaction. The temperature began to rise on 15th May, reached 107.4° on the 17th and remained between 104 and 106° for 4 more days. This subinoculation was, therefore, also negative.

Immunity test.—On 9th May, 34 days after the feeding of the mosquitoes and 20 days after the second and more marked reaction, the original sheep was injected with 1 c.c. B.T.V. A typical reaction followed. Six days later the temperature began to rise, 106.8°, the maximum, being reached the next day. A more or less elevated temperature (103-106.2°) was maintained for 6 more days.

The *result* of this experiment must be regarded as *doubtful* for the same reasons as some of the previous ones. Eight days after the feeding of the mosquitoes quite a typical fever reaction with temperatures up to 106° appeared. However, the same sheep tested for immunity 20 days later proved to be normally susceptible. Two other susceptible animals injected with blood of this sheep taken towards the end of the fever period, did not show any reaction after the injection.

D. Experiments with *Aedes hirsutus.*

Only one experiment with three specimens could be conducted with *A. hirsutus.*

Virus sheep 9.

Mosquito group 5.—Fed on virus sheep 9 during the night of 18th to 19th March. Fourth (or second to third) day of fever. Temperature 105 and 105°. 10 specimens (reared from larvae) engorged. Used for experiment 26.

Experiment 26 (B.T. 20). *3 Aedes hirsutus.*
Feeding. Interval 17 days. Sheep 33533.

On 4th April, 1932, *A. hirsutus* group 5 was fed on the above sheep and 3 specimens engorged themselves. These mosquitoes had fed 17 days previously on an infected sheep.

Reaction.—Throughout the observation period of 21 days the temperature remainder normal, 103.4° being the maximum recorded.

On 26th April the *immunity test* was applied, 1 c.c. B.T.V. being injected. The temperature rose 3 days later up to 107°, whereas the next day 105.2 and 104.6° were registered. Two days of normal temperature followed. On the 7th day the temperature rose again to 105.7 and 105°. After 2 further days of normal temperature 105 and 105.4° were recorded on the 10th day and 104.8 and 105° on the 11th, after which the temperature returned to normal, where it remained. In this case temperature reactions were noticed on the 3rd and 4th, the 7th and the 10th to 11th days. The first elevation of temperature was too early for a bluetongue reaction; the two others were not typical and very weak.

Result.—The feeding of mosquitoes was no followed by any temperature reaction at all, but notwithstanding that the experiment has to be regarded as *doubtful*, as the reaction following the immunity test was, if it is to be regarded as a reaction at all, very weak.

E. Discussion of the Results Obtained with the Vaccine Strain of Virus after one Definite Passage through Mosquitoes.

In March, 1932, a positive case of bluetongue was obtained by the injection of 60 *Aedes lineatopennis,* which had fed on an infected sheep 17-19 days previous (*vide* Experiment 10 of the first chapter). Blood of the mosquito-infected sheep was subinoculated in two other animals, and these also showed typical reactions. On

these three sheep 592 mosquitoes were fed. 446 *A. caballus*, 136 *A. lineatopennis* and 10 *A. hirsutus*. At the time of the reactions of these sheep, about the middle of March, *A. caballus* were numerous, a fair number of *A. linneatopennis* could be obtained, whereas only a few specimens of *A. hirsutus* were bred.

The mosquitoes were used in this series of experiments mainly for feeding experiments. After their feeding on one of the virus sheep the mosquitoes were kept on sugar water in cages or jars in our warm room. They were then first fed on susceptible sheep after an interval of 14 days, allowing thus sufficient time for a development of the virus in these insects. Part of the mosquitoes were later fed once again on other susceptible sheep and some were emulsified and injected subcutaneously into sheep. When fever reactions followed the feeding or injection of mosquitoes, blood was subinoculated into other sheep. All the sheep used were tested for immunity after a sufficient time had elapsed by subinoculation of 1 c.c. of the normal vaccine virus.

In all, 9 experiments were carried out, the results of which we will briefly summarize.

Aedes caballus, experiment 18, fed 16 specimens after an interval of 14-15 days. No reaction. Sheep normally susceptible. Result *negative*.

A. caballus, experiment 19, fed 25 specimens after an interval of 14-15 days. Short reaction after 6 days and 10 days later. Immunity test applied 29 days after the first reaction gave a somewhat a typical reaction of similar intensity. Subinoculations into 2 susceptible sheep were negative. Result of the experiment *doubtful*.

A. caballus, experiment 20, fed 10 specimens (the same mosquitoes as in experiment 19) after an interval of 19 days. A fairly typical reaction of medium intensity occurred 11 days later. The immunity test applied 19 days after this reaction gave a marked reaction. Subinoculations into 2 susceptible sheep failed to produce any reaction. The result of the experiment was *doubtful*.

A. caballus, experiment 21, 1 specimen, which had been used in the two preceding experiments, was injected after 35-36 days into a sheep. No reaction. Sheep normally susceptible. Result *negative*.

A. lineatopennis, experiment 22, fed 8 specimens after an interval of 14-20 days. No reaction. Sheep susceptible, reaction of the immunity test, however, somewhat atypical. Result *negative*.

A. lineatopennis, experiment 23, 10 specimens, the remainder of the preceding experiment, injected after 22 days. No definite reaction. Reaction of the immunity test not typical. Result *doubtful*.

A. lineatopennis, experiment 24, 15 specimens fed after an interval of 14-15 days. After 12 days a temperature reaction, however, not very marked, occurred. When tested 18 days later for

immunity the sheep was normally susceptible. During the short febrile reaction blood was subinoculated into 2 sheep. One animal remained normal, the other however developed a typical and marked fever reaction accompanied by slight but typical clinical symptoms after an incubation period of 5 days. There seems to be no cause for doubt as to the bluetongue character of this reaction, and furthermore, when tested 17 days after the conclusion of the reaction, the sheep proved to be totally immune. Apart from the result of the immunity test this experiment seems to be *positive* on account of the definite result in one of the subinoculated animals.

A. lineatopennis, experiment 25, fed 16 specimens, the remainder of the mosquitoes used in the preceding experiment, after an interval of 18-26 days. Eight days later a fairly typical temperature reaction appeared. However, 20 days later the sheep proved to be normally susceptible when tested for immunity. Blood taken at the end of the first febrile reaction and inoculated into 2 normal sheep gave negative results. The result of the main experiment was *doubtful*.

A. hirsutus, experiment 26, fed 3 specimens after an interval of 17 days. No reaction. Immunity test very doubtful. Result of the experiment therefore *doubtful*.

In reviewing the results of these experiments the main feature appears to be the fact that most of them are not clear cut.

The most important experiment undoubtedly is No. 24 with *A. lineatopennis*, fed after an interval of 14-15 days on a sheep. In this experiment one of the subinoculated animals, sheep 34531, showed a bluetongue reaction, which allowed no doubt as to its nature, a marked and typical temperature reaction, slight but typical clinical symptoms (the vaccine strain used never shows marked symptoms under experimental conditions) and a total immunity after the end of the reaction. The original sheep, however, showed only a short and mild reaction which produced no immunity.

The same mosquitoes were fed on another sheep 4-11 days later (experiment 25) and once more, in this case after 8 days, quite a typical reaction occurred. Also, in this sheep no immunity resulted and the subinoculations of blood (taken after the temperature had commenced to drop) were also negative.

Similar results were obtained in the experiments with *A. caballus*.

In experiment 19 after feeding a batch of mosquitoes which had been infected 14-15 days previously, 5 days later a short but quite marked temperature reaction appeared. Subinoculations, however, were negative and the sheep later proved to be normally susceptible.

The same mosquitoes, fed on another sheep 4 days later (experiment 20), gave once more the same result, quite a good febrile reaction after 11 days, no immunity and negative subinoculations.

It must be pointed out that all the sheep used in these experiments normally showed a regular temperature, and some of them a very regular one. The animals in this series were observed for at least a fortnight before use and those with somewhat irregular temperatures were discharged. The reactions therefore certainly were not normal fluctuations of the temperature.

Experiments with 16 *A. caballus* fed after 14-15 days (experiment 18), 1 specimen injected after 35-36 days (experiment 21), and 8 *A. lineatopennis* fed after 14-20 days (experiment 22) were negative. Experiments with 10 *A. lineatopennis* (No. 23) injected after 22 days and with 3 *A. hirsutus* (experiment 26) after 17 days were doubtful, although in all probability also negative. These negative or doubtful results were all obtained with mosquitoes fed on virus sheep 8 (infected by injection of mosquitoes), whereas the mosquitoes giving a positive or doubtful, but quite typical reactions, had infected themselves on virus sheep 9 and 10 (infected by sub-inoculation of blood from virus sheep 8).

It is, therefore, not impossible that part of our failures in these experiments was due to a slight degree of virulence of the virus. It is a known fact, that the virulence of the virus for sheep decreases very rapidly under laboratory conditions. We had the same experience, e.g. when injecting blood from spontaneous clinical cases into sheep in the laboratory, the course of the disease being mild and closely simulating that produced by the vaccine strain. This appears to be mainly due to the better conditions under which sheep are kept in stables than is the case under field conditions.

The small amount of virus injected into a sheep by an infected mosquito may just have been sufficient to produce a fever reaction, but it was insufficient to produce an immunity capable of resisting the comparatively massive doses normally used when injecting virus by means of the syringe.

Furthermore, the virus of this strain may have been present in the blood of mosquito-infected sheep in sufficient quantity to produce infections by subinoculation for only a relatively short time. Once present in sufficient amount in the blood, the virus would produce normal immunity as in sheep 34531 (cf. also the results of mosquito feeding on virus sheep 8, infected through mosquito injection and on virus sheep 9-10, infected through subinoculations).

These are certainly possibilities whereby our results could be explained but they do not entirely satisfy us.

Concluding this discussion we believe that we *have succeeded in transmitting a bluetongue infection by means of allowing infected specimens of* Aedes lineotopennis *to feed on susceptible sheep. The experiments, however, were not sufficiently clear cut and they do not permit of any definite conclusion about the natural transmission of bluetongue to be arrived at.*

On the other hand, the experiments certainly do not exclude *A. lineatopennis* and *A. caballus* as possible vectors.

VI. EXPERIMENTS WITH THE VACCINE VIRUS AFTER TWO (TRUE OR SUPPOSED) PASSAGES THROUGH MOSQUITOES.

In the preceding chapter we have described some experiments with a strain of the ordinary vaccine virus after a survival of 17-19 days in mosquitoes. After feeding the mosquitoes infected with this strain more or less marked fever reactions appeared in some cases, which, however, were not followed by immunity. Only in the case of one sheep infected by subinoculation of blood was a complete immunity acquired. On three of these sheep, mosquitoes were fed during the fever reaction and after an interval of at least 14 days, these were again fed on susceptible animals.

During the interval these mosquitoes were kept on sugar water in our warm room, which was only heated at night at that time as the day temperature was sufficiently high for virus development.

At the time of these experiments, viz., towards the end of the summer (7th–22nd April), *Aedes caballus* were still obtainable in sufficient numbers from one of our experimental breeding places. A fair number of *A. lineatopennis* was also present, but we lost the group of this species later through an unfortunate accident.

A. Virus Sheep.

The following three sheep, infected in experiments described in the preceding chapter, were used for infecting mosquitoes. The reactions shown by these animals were of a true bluetongue nature or at any rate practically indistinguishable from such reactions.

Virus sheep 11 = sheep 33528 from experiment 19, reacting after feeding of *Aedes caballus.*

Result.—This sheep showed a series of febrile reactions. We are here concerned with only the first of them. The temperature on 7th April, 6 days after the feeding of the mosquitoes, was 102.6-106.2°, the 8th 105.4 and 106°, the 9th 106 and 103.2° and the 10th (a.m.) 104.6°. Tested later by injection of B.T.V. this sheep was susceptible.

Virus sheep 12 = sheep 33554 from experiment 25, reacting after feeding of *A. lineatopennis.*

Result.—The mosquitoes were fed on 5th April, and 12 days later a short reaction occurred. The temperature on 17th April was 106 and 106°, the 18th 106 and 105.6°, the 19th 104.2 and 104.6°. Tested later by injection of B.T.V. the sheep had not acquired an immunity.

Virus sheep 13 = sheep 34531 from experiment 24. This sheep was injected on 14th April, 1932, with blood of sheep 32076 which had shown a slight fever reaction after the feeding of infected *A. lineatopennis.*

The temperature was normal up to 18th April, and on the 19th 103.4 and 104.8°, the 20th 103.8 and 105.4, the 21st 105.6 and 105.7°, the 22nd 104.8 and 106.6°, the 23rd 105.5 and 106.1°, the 24th (a.m.) 105.6°, the 25th 103.9 and 104.8°. The temperature then went back to normal. When tested later by injection of B.T.V. this sheep proved to be totally immune.

B. Experiments with *Aedes caballus.*

On each of the three virus sheep one group of *A. caballus* was fed. In all, 382 specimens engorged themselves. The mortality amongst the mosquitoes in this case was considerable; especially was this the case amongst the first group which were caught as adults. They died *en masse* during the period of oviposition 4 days after they fed. The other two groups retained their vitality much longer.

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With these mosquitoes 5 experiments (Nos. 27-31) were carried out and 136 specimens were fed again on susceptible sheep after an interval of 14-21 days.

Experiment 27 was carried out with mosquitoes fed on virus sheep 11; experiments 28 and 29 with specimens from virus sheep 12 and experiments 30 and 31 from virus sheep 13.

Virus sheep 11-13 and mosquito groups:

Group 21.—Fed during the night of 7th to 8th April, 1932, on virus sheep 11. First day of fever. Temperature 106.2-105.4°. 118 specimens (caught as adults) engorged. Used for experiment 27.

Group 28.—Fed during the night of 18th to 19th April on virus sheep 12. Second to third day of fever. Temperature 105.6-104.2°. 118 specimens (reared from larvae) engorged. Used for experiments 28 and 29.

Group 34.—Fed during the night of 22nd to 23rd April on virus sheep 13. Fourth day of fever. Temperature 106.6-105.5°. 121 specimens (reared from larvae) engorged. Used for experiments 30 and 31.

EXPERIMENT 27 (B.T. 25). 2 *Aedes caballus.*
Feeding. Interval 15 *days. Sheep* 34084.

During the night of 22nd to 23rd April, 1932, 2 (out of 3) *A. caballus* of group 21 were fed on this sheep. They had infected themselves 15 days before on virus sheep 11 (of experiment 19) during the first day of a febrile reaction.

Reaction.—The sheep was kept under observation for 21 days. During this period no reaction occurred, no temperature above 104° being recorded.

Immunity test.—On 14th May the sheep was inoculated with 1 c.c. B.T.V. On 19th May a short rise to 106° occurred, the temperature dropping to normal, however, the same day. On 21st May another, and this time marked, reaction began lasting 4-5 days with 107° as maximum.

Result.—This experiment was negative. After the feeding of 2 specimens no temperature reaction followed, whereas the sheep was normally susceptible.

EXPERIMENT 28 (B.T. 33). 41 *Aedes caballus.*
Feeding. Interval 15 *days. Sheep* 34550.

On 3rd May, 1932, *A. caballus* group 28 was put on sheep 34550 and 41 (out of 77) specimens engorged themselves during the night. These had fed 15 days before on virus sheep 12 (of experiment 25) during the second to third day of fever.

Reaction.—Up to 15th April, i.e. 12 days following the feed the temperature remained normal. A short rise to 105.6° lasting, however, less than 24 hours, then appeared. Thereafter another week of normal temperature followed. On 24th May, 104.6° was recorded, the temperature remained between 103.8 and 104.6° for 3 days and then dropped to normal.

Immunity test.—On 28th May, 25 days after the feeding of the mosquitoes the sheep was injected with 1 c.c. B.T.V. The following day the temperature started to rise again, 105.4 being recorded on 30th May. The temperature dropped one day later, but directly commenced rising again and fluctuated between 103.4 and 105.4° from 31st May to 4th June. On 6th May it was normal again but the following day a more typical reaction set in, lasting 5 days with 107° as the highest point of the reaction. On 17th June the sheep was discharged from observation.

Result.—It is difficult to form a definite opinion about the result of this experiment, and we have to regard it as *doubtful.* Twenty days after the feeding of the mosquitoes a temperature reaction set in; 4 days later the sheep was injected with B.T.V. The temperature remained irregular, but 10 days later a more typical reaction commenced which would appear to have been the reaction following the virus injection. On scrutinizing the temperature chart, however, the possibility of all these reactions commencing on 24th May, prior to the immunity test, being connected with one another presents itself. Furthermore, the inoculation period of what is considered to be that of the normal bluetongue reaction of the immunity test was longer than normal, viz., 10 days.

EXPERIMENT 29 (B.T. 40). 36 *Aedes caballus*.

Feeding. Internal 21 *days. Sheep* 34409.

During the night of 9th to 10th May the same mosquitoes as used in the preceding experiment were put on to sheep 34409. 49 specimens still remained and of these 36 engorged themselves. The interval was now 21 days.

Reaction. -On 17th May a short rise of temperature up to 104.8°, lasting, however, only ¼ day, occurred. 10 days of normal temperature followed. The temperature then became somewhat irregular, varying between 102.8 and 104.6°.

Immunity test.—On 10th June, a month after the feeding of the mosquitoes, 1 c.c. B.T.V. was injected. Directly after this injection the temperature rose to 107°, but had regained normal by the next morning. After 8 days a marked reaction set in lasting about 6 days with temperatures of up to 106.8° and remissions of 102°

Result.—This experiment is to be regarded as *negative*. No typical reaction followed the feeding of the mosquitoes, whereas the sheep proved to be susceptible.

EXPERIMENT 30 (B.T. 37). 40 *Aedes caballus*.

Feeding. Interval 14 *days. Sheep* 34175.

On 6th May, *A. caballus* (group 34) was put on to this sheep, and during the following night 40 (out of 61) specimens engorged themselves. These mosquitoes had fed 14 days before on virus sheep 13 (sheep 34531 from experiment 25) during the 4th day of fever.

Reaction.—During the first 12 days a normal and regular temperature was maintained (102.4-103.8°). It then began to rise, and on 22nd June 105 and 105.4° were reached. 23rd June 105.4 and 102.1° were recorded, the 24th (a.m.) 104.8°, the 25th 104.6 and 105°, the 26th 104.2 and 105°, the 27th 105 and 104.3° and the 28th (a.m.) 105.2°.

Immunity test.- The same day, 28th May, the sheep was injected subcutaneously with 1 c.c. B.T.V. That afternoon the temperature rose to 106.4, a temperature which was maintained until the following morning. It then dropped to normal and began to rise again on 5th June, 8 days after the injection, reaching 106.8°, the highest point, on 9th June. Four days later the reaction came to an end.

The *result* of the experiment is to be regarded as *doubtful*. 13-16 days after the feeding of 40 infected mosquitoes a marked fever reaction set in. At its highest point, owing to an unfortunate error, the sheep was tested for immunity and 8-9 days later a similar reaction occurred. It is possible in any case, that the first reaction was due to a bluetongue infection transmitted by the mosquitoes which, however, did not confer or had not then conferred to the sheep a sufficient degree of immunity to resist without any reaction the subsequent virus injection.

EXPERIMENT 31 (B.T. 41). 17 *Aedes caballus*.

Feeding. Interval 20 *days. Sheep* 34423.

On 12th May the remaining specimens of *A. caballus* (group 34), which had already been used in the preceding experiment, were put on to sheep 34423 and during the night 17 (out of 25) specimens fed

Reaction.—On 14th May, two days after the feeding, a temperature of 106.2° was recorded, but the following day it commenced to drop, and remained normal for 9 days, after which a somewhat irregular course was noticed, temperatures of 104-104.8° being noticed on 24th, 29th, 31st May, 1st and 4th June.

Immunity test.—On 8th June, 27 days after the feeding of the mosquitoes, the sheep was injected subcutaneously with 1 c.c. B.T.V. 15th June, 7 days later, the temperature began to rise. The reaction which was of medium intensity, lasted 3-4 days, the highest temperature recorded being 106.4°.

The *result* of this experiment was *negative*. After the feeding of the mosquitoes no typical reaction occurred, whereas the sheep proved to be susceptible.

C. Experiments with *Aedes lineatopennis*.

When these experiments were undertaken in April *Aedes lineatopennis* was very difficult to obtain. Only one group could be fed on one of the virus sheep and this was again fed 15 days later on a susceptible sheep. Shortly afterwards this group was accidently lost.

Virus sheep 11 was used and *mosquito group* 16.—Fed during the night of 7th to 8th April on virus sheep 11. First day of fever. Temperature 106.2-105.4°. 75 specimens (caught as adults) engorged. Used for experiment 32.

Experiment 32 (B.T. 26). *Aedes lineatopennis.*
Feeding. Interval 15 days. Sheep 34797.

On 22nd April, 1932, *A. lineatopennis* group 16 was put on to sheep 34797 and during the night 10 (out of 20) specimens fed. They had their initial feed 15 days previously on sheep 33528 during the first day of a febrile reaction.

No real *reaction* followed the feeding of these mosquitoes during an observation period of 23 days. Only once, 4 days after the infecting feed of the mosquitoes, was a temperature in excess of 104° noticed (104.4°). This, however, was maintained for less than 24 hours.

Immunity test.—On 16th May the sheep was injected subcutaneously with 1 c.c. B.T.V. 24th May, 8 days later, the temperature began to rise, reaching 106.6°, the maximum of the reaction, 2 days later. The reaction lasted 3-4 days, and although not very marked, was quite typical.

The *result* of this experiment was *negative*, no typical reaction following the feeding of the mosquitoes on a sheep which, when tested for immunity, proved to be susceptible.

D. Results of Experiments with the Vaccine Virus after two (supposed) Passages through Mosquitoes.

In April, 1932, we obtained fever reactions in sheep after the feeding of mosquitoes which had infected themselves on a sheep showing a bluetongue reaction after the injection of mosquitoes or on two other animals injected with blood of the first sheep. These fever reactions resembled more or less true bluetongue reactions, but they did not produce any immunity. Only one sheep, subinoculated with blood taken from one of these animals during the fever period, showed a typical reaction combined with slight clinical symptoms and later a complete immunity.

On three of these sheep, two original and one of the subinoculated cases, 382 *Aedes caballus* and 75 *A. linneatopennis* were fed. These mosquitoes were kept as usual on sugar water in jars or cages in our warm room after the feed. Following an interval of 14 days or longer, the mosquitoes were fed on new susceptible sheep.

In all, 6 experiments were conducted, 5 with *A. caballus* and 1 with *A. lineatopennis*. In the experiments with *A. caballus* all three virus sheep were used; in the case of the latter species only one sheep, viz., one of the original cases.

Aedes caballus, experiment 27, was carried out with mosquitoes fed on virus sheep 11, one of the original cases. 2 specimens were fed after an interval of 15 days with *negative* results.

For *Aedes caballus*, experiment 28, mosquitoes from virus sheep 12 were used, also one of the original cases showing no subsequent immunity. 41 specimens fed after 15 days. The result was *doubtful*, as a few days before the sheep was injected for testing the

immunity a fever reaction set in, continuing through the incubation period of the immunity reaction, which, besides that, showed a normal course.

A. caballus, experiment 29, was carried out with the same mosquito group, of which 36 specimens fed for the second time after an interval of 21 days. In this case, however, the result was without doubt *negative;* no reaction followed the feeding of the mosquitoes, whereas the sheep was normally susceptible.

A. caballus, experiment 30, was made with mosquitoes which had fed on the subinoculated sheep showing a true bluetongue reaction followed by complete immunity. 40 specimens fed after an interval of 14 days. 13-16 days later a marked reaction set in. Before this reaction was closed unfortunately the immunity test was applied and this showed the normal course. The result therefore was *doubtful*.

A. caballus, experiment 31, was made with the same group of mosquitoes (17 specimens) after an interval of 20 days. The result was *negative*.

A. lineatopennis, experiment 32, with 10 specimens after an interval of 15 days. Result *negative*.

In all these experiments 5 susceptible sheep were bitten 139 times by infected *A. caballus* after intervals ranging between 14 and 21 days and by 10 *A. lineatopennis* after 15 days. Four of these experiments were certainly negative, whereas in two the results were doubtful. Especially in one of these cases (experiment 30), in which 40 *A. caballus* were fed after 14 days, there is a definite possibility that the experiment was really positive.

It must be remembered that these mosquitoes were fed on three virus sheep, of which only one was definitely positive. With mosquitoes fed on this sheep, experiment 30 was conducted. No further discussion of the results seems to be necessary and we may refer to the preceding chapter.

VII. EXPERIMENTS WITH A STRAIN FROM IXOPO.

At the beginning of April, 1932, through the courtesy of the Government Veterinary Officer, we received a quantity of preserved blood from a natural case of bluetonge from Ixopo (Natal). Our hope that with this material clinical cases of bluetongue could be produced and that in future we would not have to rely any more on only more or less typical temperature reactions as produced by the vaccine strain of bluetongue was, however, not realized. Two sheep were injected with this material, but both showed only very slight reactions lasting less than 48 hours in each case and showing no clinical symptoms. The virulence, therefore, was less than that of the ordinary vaccine strain. Mosquitoes were fed on these two sheep but thereafter the strain was abandoned.

At the time of the short febrile reaction in these sheep *Aedes caballus* was the only mosquito species at our disposal in large numbers. In all, 413 specimens engorged themselves. They were

kept in our warm room in the usual way after this feeding, but their numbers diminished rapidly owing to an extraordinary high mortality amongst them.

Apart from this species a few *A. lineatopennis* were fed and also some *A. durbanensis*, which appeared during that time in one of our experimental breeding places.

The experiments were conducted in the usual manner, the infected mosquitos being refed on susceptible sheep after an interval of at least 14 days.

A. VIRUS SHEEP.

The following two sheep were injected with the Ixopo strain:

Virus sheep 14 = sheep 31553. Injected on 4th April, 1932, subcutaneously with 2 c.c. preserved blood of a natural case.

Result.—The temperature remained normal or nearly normal up to 10th April. On the 11th (a.m.) it was 105°, on the 12th during the morning 107° and during the afternoon 104°. It then remained between 103 and 104.6° for 6 days and later on between 102 and 104°. The actual febrile reaction was, therefore, very short, lasting less than 48 hours.

Virus sheep 15 = sheep 31570. Injected together with the preceding sheep with the same material.

Result.—The temperature remained normal up to 10th April, when 103.8° was recorded during the morning. On the 11th it registered 107 and 106.8°, the 12th 105.8 and 103.4° and from the next day onwards normal temperatures were maintained, ranging between 102 and 104.6°. In this case as well the reaction, although definite, was very short, less than 48 hours.

B. EXPERIMENTS WITH *Aedes caballus*.

On both virus sheep a batch of *A. caballus* was `led during the nights following 11th and 12th April. Owing to the very short duration of the reaction, the last two batches were actually fed after the temperature had already returned to normal.

During the first night 121 specimens were fed, of which 17 survived at the end of 15 days. Of 252 fed during the second night 23 survived. 24 days later only 6 out of the four batches were left. The mortality was extremely high in this case.

The mosquitoes were fed on three susceptible sheep; the two batches fed on the virus sheep during the first night were refed on the first of these three animals after an interval of 15 days, the last two groups were refed at the same time on the second sheep and the remaining specimens of the lots together were fed on the last animal at the end of 24 days. After 45-46 days 2 specimens were still alive and they were injected into a sheep.

The two virus sheep 14 and 15 were used and the following mosquito groups:

Group 22.—Fed on virus sheep 15 during the night of 11th to 12th April. First to second day of fever. Temperature 106.8-105.8°. 95 specimens (reared from larvae) engorged. Used for experiments 33, 35 and 36.

Group 23.—Fed on virus sheep 14 during the same night. First day of fever. Temperature 107.8°. 26 specimens (reared from larvae) engorged. Used for experiments 33, 35 and 36.

Group 24.—Fed on the same virus sheep during the following night. First day after the end of the febrile reaction. Temperature 104-104.2°. 150 specimens (reared from larvae) engorged. Used for experiments 34-36.

Group 25.—Fed on virus sheep 15 during the same night. First day after the end of the febrile reaction. Temperature 103.4-102.6°. 142 specimens (reared from larvae) engorged. Used for experiments 34-36.

EXPERIMENT 33 (B.T. 28). 13 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34758.

On 26th April, 1932, the remaining specimens of the combined groups 22 and 23 were put on to sheep 34758 and 13 (out of 17) specimens engorged themselves during the ensuing night. These mosquitoes had fed on an infected sheep 15 days previously at the peak of a short febrile reaction.

Reaction.—The temperature of this sheep during the week preceding the feeding of the mosquitoes had never exceeded 103°. 7 days after the feeding of the mosquitoes, however, a temperature of 103.4° was noticed, the following morning 103.8° was recorded and for the following 5 days temperatures higher than 103° were frequently seen. Thereafter the temperature became somewhat irregular with unmaintained rises to 104.2 and 104.4° on 19th and 27th May, the day before the application of the immunity test.

Immunity test.—On 28th May, 32 days after the feeding of the mosquitoes, 1 c.c. blood of virus sheep 14, taken on 12th April, was injected subcutaneously. The temperature commenced rising immediately either owing to the virus injection or as a continuation of the slight rise noticed on the previous day, and on 30th May, 105.2° was reached. An evening remission to 102.6° occurred on this date, but on the 31st the temperature commenced rising again and the reaction continued, reaching 105.6, its highest point, on 4th June. The reaction came to an end shortly after this.

Result.—We regard this experiment as *negative*, notwithstanding the fact that something like a febrile reaction commenced just before the application of the immunity test. The reaction following the immunity test was of medium intensity, but a more marked fever could not be expected with this strain owing to its low virulence.

EXPERIMENT 34 (B.T. 30). 14 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34606.

On 27th April, 1932, the surviving specimens of *A. caballus* group 24 and 25 were put on to sheep 34606 and during the night 14 (out of 23) specimens engorged themselves. These mosquitoes had fed 15 days previously on the day following the febrile reactions of the two sheep infected with the Ixopo strain.

Reaction.—During the first 8 days the temperature remained normal (102-103.4°). On 6th May, it rose to 104.8°, returned to normal the next day, but showed a further slight elevation (104 and 104.2°) on the following two days. Thereafter it remained practically normal up to 27th May, showing only two slight elevations (104 and 103.8°), lasting in each case ½ day.

Immunity test.—On 28th May the sheep was injected subcutaneously with 1 c.c. blood of sheep 31553, one of our virus sheep. Four days later, on 1st June, a sudden rise to 105.6° occurred, which lasted, however, for only ½ day. A further rise occurred 2 days later and the temperature remained between 105 and 106.8° for 3 days. It then dropped again, but remained more elevated than usual (104-105.4°) for the next ten days.

The *result* of this experiment was *negative*. The reaction occurring 9 days after the feeding of the mosquitoes was not sufficiently definite to be of any importance. The immunity test was of medium intensity but clear enough.

EXPERIMENT 35 (B.T. 35). 4 *Aedes caballus*.
Feeding. Interval 23-24 *days. Sheep* 34390.

On 6th May, 1932, the remaining mosquitoes of the two preceding experiments were used again and 4 out of 6 specimens engorged themselves. They had ted 23-24 days before on two infected sheep during the top point of the febrile reactions and during the day after the end of this reaction.

Reaction.—To start with the temperature remained normal for the first 14 days (101.6-103.2°). A short rise to 104.6° then occurred, whereafter another period of normal temperatures followed.

Immunity test.—On 28th May, 22 days after the feeding of the mosquitoes, the sheep was injected with 1 c.c. blood of sheep 31533, the same material as used in the preceding tests. Five days later a definite rise in temperature occurred, 106.8° being reached 2 days later. The fever period lasted 4-5 days, the reaction being typical and quite marked.

The *result* of this experiment was *negative*. No reaction followed the feeding of the mosquitoes, and the sheep was normally susceptible.

EXPERIMENT 36 (B.T. 40). 2 *Aedes caballus.*
Injection. Interval 45-46 days. Sheep 34360.

On 27th May 2 specimens of the combined groups 22-25 were still alive and they were injected into sheep 34360 on that day. They had had their original feed on a virus sheep 45-46 days previously.

Reaction.—A normal temperature was maintained for 7 days up to 3rd June. The temperature was on the 4th 103.6 and 105.6°, the 5th (a.m.) 106°, but from the day thereafter it was normal again.

Immunity test.—On 20th June, 24 days after the injection of the mosquitoes, the sheep was subcutaneously injected with 1 c.c. blood from sheep 31570. A short, but quite typical reaction commenced to appear 7 days later, lasting about 3 days, with 107.4° as maximum.

Result.—The experiment has to be regarded as *negative*. The short reaction following the injection of mosquitoes cannot be considered as a bluetongue reaction and the sheep proved to be susceptible later when tested for immunity.

C. EXPERIMENTS WIHT *Aedes lineatopennis.*

At the time that these experiments were carried out *A. lineatopennis* was very difficult to obtain and not more than 10 specimens could be induced to engorged themselves on the virus sheep. Of these a few specimens were refed on a susceptible sheep after a period of 15 days and longer. After 45 days 2 specimens were still alive and they were injected into a sheep.

Virus sheep 14 and 15 were used and mosquito groups:

Group 17.—Fed on virus sheep 15 during the night of 11th to 12th April. First or second day of fever. Temperature 106.8-105.8°. 3 specimens (reared from larvae) engorged. Used for experiment 32.

Group 18.—Fed on virus sheep 14 during the same night. First day of fever. Temperature 107.8°. 3 specimens (reared from larvae) engorged. Used for experiment 37.

Group 19.—Fed on the same virus sheep one night later. First day after the end of the febrile reaction. Temperature 104-104.2°. 4 specimens (reared from larvae) engorged. Used for experiments 37 and 38.

EXPERIMENT 37 (B.T. 27). *Aedes lineatopennis.*
Feeding. Interval 15-29 days. Sheep 34053.

During the night of 26th to 27th April the combined groups 17 and 18 were put on to this sheep, but only 1 (out of 3) specimens fed. The following night batch 19 was put on and 2 (out of 4) mosquitoes fed. The same lot of mosquitoes was again put on to the sheep during the night of 10th to 11th May and 2 specimens probably fed. This point was not ascertained with certainty, however. During the following night 1 specimen (out of 2) from groups 17-18 engorged itself.

The sheep was, therefore, bitten by 3 specimens after a period of 15 days, by 2 (?) after 27, and by 1 specimen after 29 days.

Reaction.—No definite fever reaction followed the feeding of these mosquitoes. From time to time (29th April, 21st, 28th May, 3rd, 4th June) short elevations varying between 103.8 and 105° were noticed, but they were irregular and not connected with one another as a reaction.

Immunity test.—On 8th June, 43 days after the first, and 29 days after the last, feeding of the mosquitoes the sheep was injected subcutaneously with 1 c.c. blood of sheep 31570 (virus sheep 15). On 14th June, 6 days later, the temperature rose, reaching 106° the same day, 106.4° the following, and 107° the day thereafter, however, with daily remissions to 104°. On 12th June, the reaction came to an end.

The *result* of this experiment was *negative*. No reaction followed the feeding of the mosquitoes. The reaction of the immunity test was not very marked, probably on account of the low virulence of this strain.

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EXPERIMENT 38 (B.T. 41). *2 Aedes lineatopennis.*
Injection. Interval 45 days. Sheep 34410.

On 27th May, 2 *A. lineatopennis* from group 2 were still alive and they were injected on that day together with 1 specimen infected with the Kameelfontein strain into sheep 34410.

Reaction.—No fever reaction followed the injection of the mosquitoes. The highest temperature being registered during the period of 23 days was 104.2°.

Immunity test.—On 20th June, the sheep was injected subcutaneously with 1 c.c. blood from sheep 31570 (virus sheep 15). No reaction, however, followed during the next 20 days, the temperature not exceeding 104°. On 11th July the sheep was injected with 1 c.c. of the same material and this time, after 5 days, a typical reaction commenced, lasting 3-4 days, with 106.5° as maximum.

Result.—The experiment has to be regarded as *negative*. The result of the immunity test was, however, very remarkable. The first injection was absolutely negative, whereas a subsequent injection with the same material was followed by a typical reaction.

D. EXPERIMENT WITH *Aedes durbanensis.*

With this relatively rare species only a single experiment with 1 specimen could be carried out.

Virus sheep 14 was used and mosquito group:

Group 2.—Fed on virus sheep 14 during the night of 12 to 13th April. First day after the end of the febrile reaction. Temperature 104-104.2° 2 specimens (reared from larvae) engorged. Used for experiment 39.

EXPERIMENT 39 (B.T. 29). *Aedes durbanensis.*
Feeding. Interval 16 days. Sheep 34567.

During the night of 27th to 28th April the 2 *A. durbanensis*, which had fed 16 days previously, were put on to sheep 34567 and one specimen fed.

Reaction.—The sheep was kept under observation for 31 days, but no temperature reaction occurred, 104° being exceeded only once for ⅓ day.

Immunity test.—On 28th May, the sheep was injected subcutaneously with 1 c.c. blood of virus sheep 14. After 7 days the temperature began to rise, reaching 107.2°, its highest point, on 5th June. Then it began to drop, but was followed by clinical symptoms, red coronets, mucous discharges from the mouth, and excoriations of the gums.

The *result* of this experiment is clearly *negative*. No reaction followed the mosquito feeding, whereas the immunity test showed a marked reaction with clinical symptoms. The appearance of these symptoms was quite unexpected, as the original sheep, from which the virus was derived, showed only a slight reaction and other immunity tests carried out with the same material gave only reactions of medium intensity.

E. RESULTS OF EXPERIMENTS WITH THE IXOPO STRAIN
OF BLUETONGUE.

At the beginning of April, material from a natural case of bluetongue was obtained from Ixopo. When injected into two sheep only very slight reactions, less than those with the ordinary vaccine virus, were obtained and therefore only a limited number of experiments were carried out.

In all, 413 *Aedes caballus*, 10 *A. linneatopennis* and 2 *A. durbanensis* engorged themselves either during or directly after the short febrile reaction shown in the virus sheep. After this feed the mosquitoes were kept in our warm room on sugar water in cages or jars following the usual method. They were refed after an interval of at least 15 days on susceptible sheep, and after 1½ months

the remaining specimens were injected into sheep. Seven experiments were carried out and in each case after a sufficient period had elapsed the immunity was tested by subcutaneous injections with the same strain of virus.

In these experiments the following results were obtained:—

Aedes caballus, experiment 33. After an interval of 15 days 13 specimens fed. Result *negative*.

A. caballus, experiment 34. After the same interval 14 specimens fed. The result was also *negative*.

A. caballus, experiment 35, was carried out with the remaining mosquitoes of the two preceding experiments. 4 specimens fed after an interval of 23-24 days. Result *negative*.

A. caballus, experiment 36, 2 specimens injected after an interval of 45-46 days. Result *negative*.

A. lineatopennis, experiment 32, 3 specimens fed after a period of 15 days, 2 (uncertain) after 27 and 1 specimen after 29 days. Result *negative*.

A. lineatopennis, experiment 28, 2 specimens injected after 45 days. Result *negative*.

A. durbanensis, experiment 39. Only 1 specimen fed after an interval of 16 days. Result *negative*.

In these 7 experiments 27 *A. caballus* fed after a period of 15 days, and 4 after 23-24 days, 3 *A. lineatopennis* after 15, 2 (?) after 27, and 1 after 29 days, and 1 *A. durbanensis* after 16 days, 2 *A. caballus* and 2 *A. lineatopennis* were injected after 45-46 days. All experiments were negative. The virus sheep on which these mosquitoes had taken their original feed, showed, however, only an extremely mild and short reaction, and part of the mosquitoes had actually fed when the reaction had already (earlier than could have been expected) come to an end. The conditions were, therefore, very unfavourable for conducting transmission experiments and may have influenced their results.

In one of the experiments the injection of virus failed to give an infection, whereas a subsequent injection of the same material gave a typical reaction.

VIII. EXPERIMENTS WITH A STRAIN FROM KAMEELFONTEIN.

At the beginning of April, 1932, nearly at the same time as the Ixopo strain, material from another natural case of bluetongue, which had occurred in lambs on the farm Kameelfontein was obtained through the courtesy of the Extension Officer of the Pretoria District. The blood was injected into several sheep and infections of medium and marked intensity were produced, resembling very much the ordinary vaccine strain reactions. By means of further subinoculations in one case at least clinical symptoms were obtained. The strain could be regarded as suitable,

although our hope of getting a strain giving clear cut reactions and regularly slight clinical symptoms at least, was not fulfilled.

As in the case with the Ixopo strain *Aedes caballus* was the only species available in larger numbers at the time we obtained the Kameelfontein strain. In all, 402 specimens engorged themselves on the virus sheep. Thereafter they were kept in the usual way in cages in our warm room, till they were refed on susceptible sheep. The mortality amongst the mosquitoes during the period between these two feeds was very considerable.

Only a few specimens of *A. linneatopennis* were at our disposal, and of these 12 could be induced to feed.

The experiments were conducted in the ordinary manner. The mosquitoes were refed on susceptible sheep after an interval of at least 14 days and afterwards the immunity of the sheep was tested by injection of the same strain of virus.

A. VIRUS SHEEP.

In all, 5 sheep were injected with the Kameelfontein strain, and of these the following 3 were used for feeding mosquitoes on.

Virus sheep 16=sheep 31717. Injected on 5th April, 1932, subcutaneously with 2 c.c. preserved blood from the original case at Kameelfontein.

Result.—The temperature remained normal up to 11th April. On the 12th it was 106 and 105.2°, the 13th 106.2 and 105°, the 14th 105.8 and 105°, the 15th 106 and 105°, the 16th 105.2 and 105.2°, and the 17th (a.m.) 104.8°. From the next day onwards normal temperatures were maintained. The reaction lasting 6 days was marked.

Virus sheep 17=sheep 32345 was injected together with the preceding animal with the same material.

Result.—The temperature remained normal or nearly normal (not exceeding 104.5°) up to 15th April. On the 16th it was 103.7 and 105.1°, the 17th (a.m.) 104.5°, the 18th 107.6 and 108.2°, the 19th 106.8 and 106.6°, and the 20th 105 and 104.4°. Thereafter a normal temperature was regained. Notwithstanding a maximum of 108.2°, the reaction was only of medium intensity. The incubation period, lasting 11 days, was longer than usual.

Virus sheep 18=sheep 34498 was injected on 13th April subcutaneously with 1 c.c. blood, taken from virus sheep 34498 during the fever reaction.

Result.—A normal temperature was maintained up to 17th April. On the 18th it was 103.8 and 105.1°, the 19th 105.4 and 106.7°, the 20th 105.8 and 107°, the 21st 107 and 108.2°, the 22nd 107 and 104.5°, the 23rd 104.1 and 104.2°, the 24th (a.m.) 104.7°, the 25th 105 and 105°, the 26th 103.4 and 103.3°, and the 27th 105°, when the animal was killed. This sheep showed quite a severe reaction accompanied by clinical symptoms, red coronets and discharges from the nostrils. During the last days it showed a markedly ill appearance.

B. EXPERIMENTS WITH *Aedes caballus*.

On all three virus sheep batches of *A. caballus* were fed, in all 402 specimens. One batch from each sheep was used for the experiments, of which 5 were carried out. The mosquitoes were refed on the susceptible sheep for the first time after 15 days and part of them for a second time 5-10 days later.

Experiments 40 and 41 were carried out with mosquitoes infected on virus sheep 16, experiments 42-43, with specimens from sheep 17 and the last experiment with mosquitoes fed on virus sheep 18.

The following mosquito groups were used:—

Group 26.—Fed on virus sheep 16 during the night of 14th to 15th April. Third to fourth day of fever. Temperature 105-106°. 45 specimens engorged (reared from larvae). Used for experiments 40 and 41.

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Group 27.—Fed on virus sheep 17 during the night of 18th to 19th April. Third day of fever. Temperature 108.2-106.8°. 89 specimens (reared from larvae) engorged. Used for experiments 42-43.

Group 33.—Fed on virus sheep 18 during the night of 21st to 22nd April. Fourth day of fever. Temperature 108.2-107.3°. 30 specimens (reared from larvae) engorged. Used for experiment 47.

EXPERIMENT 40 (B.T. 31). 15 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34587.

On 29th April, *A. caballus* group 26 was put on sheep 34587 and 15 (out of 32) specimens engorged themselves during the following night. They had fed 15 days previously on virus sheep 16 during the third to fourth day of fever.

Reaction.—During an observation period of 28 days no reaction followed, the highest temperature registered being 103.8°.

Immunity test.—On 28th May, the sheep was injected subcutaneously with 1 c.c. blood from sheep 34498 (virus sheep 18). A marked reaction commenced 4 days later, lasting 6-7 days, with 107.8° as maximum.

The *result* of this experiment was clearly *negative*. No reaction followed the feeding of the mosquitoes, and the sheep afterwards proved to be normally susceptible.

EXPERIMENT 41 (B.T. 38). 4 *Aedes caballus*.
Feeding. Interval 25 *days. Sheep* 34100.

On 9th May, the remaining specimens of the same batch of *A. caballus* were fed on sheep 34100 and 4 (out of 12) specimens engorged themselves. The mosquitoes had had their original feed 25 days previously.

Reaction.—A normal temperature was maintained for 14 days. On 24th May, 105° was registered, but the following morning the temperature had dropped again. Thereafter it remained somewhat unstable, 104.8° being noticed on 2nd June, and 105° on 5th. These high temperatures were, however, not maintained for more than one day.

Immunity test.—On 10th June the sheep was injected with 1 c.c. blood from sheep 34498 (virus sheep 18). 11 days of normal temperature followed. On 22nd June a sudden rise up to 105° occurred, lasting, however, only ½ day and followed by 4 days of normal temperature. On 27th May, 17 days after the injection, the bluetongue reaction started at last, lasting 7 days with 107.3° as maximum.

Result.—The experiment has to be regarded as *negative*, as only a few short and unmaintained rises of temperature occurred after the feeding of the mosquitoes. The reaction of the immunity test was somewhat atypical through the exceptionally long incubation period.

EXPERIMENT 42 (B.T. 32). 31 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34464.

On 3rd May *A. caballus* group 27 was fed on sheep 34464 and the following night 31 (out of 36) specimens engorged themselves. These mosquitoes had fed on virus sheep 17 during the third day of the bluetongue reaction.

No reaction at all followed the feeding of the mosquitoes during an observation period of 24 days, 104° being the highest temperature registered.

Immunity test.—On 28th May 1 c.c. blood from sheep 34498 was injected subcutaneously and 5 days later a typical bluetongue reaction followed, lasting 5 days with 107.8° as maximum.

The *result* of this experiment was clearly *negative*.

EXPERIMENT 43 (B.T. 39). 9 *Aedes caballus*.
Feeding. Interval 21 *days. Sheep* 34184.

On 5th May, the remaining specimens of the same batch of *A. caballus* used in the preceding experiment were put on sheep 34184 and 9 (out of 12) specimens fed. These mosquitoes had taken their initial feed 21 days previously

No *reaction* followed the feeding of the mosquitoes, 104° being the highest temperature registered during an observation period of 29 days.

Immunity test.—On 8th June, the sheep was injected with 1 c.c. blood from sheep 34498. A marked reaction, lasting 4-5 days with 108° as maximum occurred after a prolonged incubation period of 9 or 12 days.

The *result* of this experiment was also *negative.*

EXPERIMENT 44 (B.T. 36). 4 *Aedes caballus.*
Feeding. Interval 15-20 days. Sheep 34045.

On 6th May *A. caballus* group 33 was put on sheep 34045 and 3 (out of 12) specimens fed during the following night. The remainder of the same batch were fed again on the same sheep during the night from 11th to 12th May and 1 (out of 3) specimens fed. The interval was in the first case 15 and in the second 20 days. The mosquitoes had had their original feed on virus sheep 18 during the fourth day of fever.

No *reaction* followed during an observation period of 37 days after the first feeding of the mosquitoes. A temperature of 104° was reached or slightly exceeded on several occasions, however, only for short periods, the sheep generally running an elevated temperature.

Immunity test.—On 13th June 1 c.c. blood of sheep 34498 was injected subcutaneously. A reaction of medium intensity commenced 9 days later, lasting about 3 days with 107° as maximum.

The *result* was *negative*, as in the preceding experiments.

C. EXPERIMENTS WITH *Aedes lineatopennis.*

With this species, which had become rare during the time of these experiments, only two experiments could be carried out, one with feeding after an interval of 14-21 days, and the other by injection of 1 specimen being left after 38 days.

Virus sheep 18 was used and the mosquito group:

Group 20.—Fed on virus sheep 18 during the night of 19th to 20th April. Second day of fever. Temperature 106.7-105.8°. 12 specimens engorged (reared from larvae). Used for experiments 45 and 46.

EXPERIMENT 45 (B.T. 34). 6 *Aedes lineatopennis.*
Feeding. Interval 14-21 days. Sheep 34329.

During the night of 3rd to 4th May, *A. lineatopennis* group 20 was fed on sheep 34329 and 5 (out of 7) specimens engorged themselves. The remaining mosquitoes of the same batch were refed 7 days later, 10th to 11th May, and 1 specimen took up blood. The first five specimens had fed 14 days and the last specimen 21 days after the initial feed on virus sheep 18.

Reaction.—A normal temperature was maintained for 22 days after the first feeding of mosquitoes. Then a short rise to 105° occurred, lasting only ½ day, whereafter the temperature remained somewhat elevated, exceeding 104°, however, only once for ½ day.

Immunity test.—On 10th June. 38 days after the first feeding of the mosquitoes, the sheep was injected subcutaneously with 1 c.c. blood from sheep 34498 (virus sheep 18). Ten days later a marked reaction appeared, lasting 5 days with 107° as maximum.

The *result* of this experiment was *negative.* The slight temperature reaction noticed was not typical at all and the sheep proved afterwards to be normally susceptible.

EXPERIMENT 46 (B.T. 41). 1 *Aedes lineatopennis.*
Injection. Interval 38 days. Sheep 34410.

On 27th May, 1 *A. lineatopennis* of group 20 was still alive and it was injected (with two other specimens fed on a sheep infected with Ixopo virus) into sheep 34410.

The *result* of the experiment was *negative.* The reaction and the immunity test have been described already under experiment 38.

D. Results of the Experiments with the Kameelfontein Strains of Bluetongue.

At the beginning of April, material from a case of bluetongue, which had occurred in a lamb at Kameelfontein (Transvaal) was obtained. It promised to be a suitable strain for our purposes, as at least in one of the injected animals slight clinical symptoms appeared.

The season, however, was already nearing its end, and *Aedes caballus* was the only species obtainable in large numbers, breeding in our experimentally flooded breeding places. Besides this species only a very limited number of *A. lineatopennis* could be obtained.

In all, 402 *A. caballus* and 12 *A. lineatopennis* were fed on the virus sheep. Unfortunately the mortality amongst the former species was considerable. The mosquitoes were kept in our warm room on sugar water as usual. After an interval of at least 14 days they were fed on susceptible sheep and part of them refed after a longer period. One specimen of *A. lineatopennis* was injected into a sheep more than 1 month later. All sheep were afterwards tested for immunity by injections with the same strain of virus.

The following results were obtained in these experiments.

Aedes caballus, experiment 40. 15 specimens fed after 15 days. Result *negative*.

Aedes caballus, experiment 41. After 25 days 4 specimens belonging to the same batch as those in the preceding experiment, fed. Result *negative*.

A. caballus, experiment 42. After an interval of 15 days 31 specimens fed. Result *negative*.

A. caballus, experiment 43. After 21 days 9 specimens belonging to the same group as those of experiment 42, fed. Result *negative*.

A. caballus, experiment 44. After an interval of 15 days 3 specimens and after 20 days 1 specimen belonging to the same group fed. Result *negative*.

A. lineatopennis, experiment 45. After 14 days 5 specimens and after 21 days 1 specimen fed. Result *negative*.

A. lineatopennis, experiment 46. Injected after 38 days 1 specimen. Result *negative*.

In these 7 experiments 49 *A. caballus* fed after 15 days, 10 after 20-21, and 4 after 25 days, 5 *A. lineatopennis* after 14 and 1 after 21 days, whereas one specimen of the latter species was injected after 38 days. *All experiments were negative.*

The experiments with *Aedes caballus* were carried out with sufficiently large numbers. The strain, which had shortly before been isolated from a natural case of bluetongue, was virulent enough. The result, therefore, must have a definite value.

DISCUSSION OF THE RESULTS OBTAINED IN BLUETONGUE EXPERIMENTS.

During the summer 1931/32 a number of experiments with bluetongue in sheep was carried out at Onderstepoort together with work on horsesickness.

The epizootological evidence at hand pointed out that the transmission of both diseases must be very similar and that most likely mosquitoes were involved in the transmission. It must be stated, however, that these epizootological evidences—the non-contagious character of the disease, its restriction mainly to the summer months, the correlation between the amount of rain and the number of cases, the protection afforded by stables at night—are somewhat meagre, fitting well, however, with the assumption of mosquitoes being the carriers.

During the same time a mosquito survey was carried out at Onderstepoort, the results of which have been described in the first paper of this series. It was found that amongst the mosquitoes some *Aedes* species best fulfilled the epizootological requirements. These species are limited to the summer months, and their numerical appearance depends more than that of any other genus on the amount of rain. *Aedes caballus, A. lineatopennis* and *A. hirsutus,* were regarded as the most suitable transmitters, *A. vittatus, A. dentatus* and some other species as possible transmitters of secondary importance.

No transmission experiments have been undertaken before with this disease.

STRAINS OF BLUETONGUE VIRUS.

Three different strains were used for these experiments, most of which were carried out with the ordinary laboratory vaccine strain, as the first material from natural cases was only obtained at the beginning of April.

The vaccine strain had been isolated from a natural case in February, 1927, and had passed in the meantime through 48 generations. In 86 per cent. of the sheep temperature reactions were shown after injections with this strain, of which 20 per cent. had to be regarded as slight reactions. Only 8 deaths had occurred in the early generations, and from the 12th generation onwards no clinical symptoms were recorded. Temperature reactions were, therefore, the only guide for the interpretation of the results in our work, and in a number of experiments no definite opinion as to the result could be reached.

At the beginning of April, 1932, material from two natural cases of bluetongue was received, one from Ixopo, Natal, and the other from the farm Kameelfontein in the Pretoria District. At that time the bluetongue season was already nearing its end. All efforts to get material from spontaneous cases earlier in the season had failed.

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Through injection of the Ixopo strain in the first generation only mild reactions were obtained, whereas the Kameelfontein material gave infections of medium to marked intensity, and in one of the further subinoculations even slight clinical symptoms.

Experimental Animals.

The sheep used for these experiments came from a known bluetongue-free area of the Cape Province. It is very exceptional to find an immune animal amongst these sheep. To be as safe as possible, however, all sheep were tested for immunity after the experiment had been finished with the same strain as was used for infecting the respective mosquitoes.

The *mosquitoes* used in the experiment were obtained by catching the larvae and pupae or the adults in the field.

They were fed on the infected and later on the susceptible sheep in small cages covered with mosquito-netting. These cages were fixed on clipped areas of the skin by pieces of tape, attached to the surrounding wool. They were covered with a piece of wet cotton wool to ensure a sufficient degree of humidity inside the cages.

The mosquitoes were kept between the feedings in a warm room on sugar water in jars or cages. In order to ensure that a sufficient degree of humidity was obtained, the jars were placed in larger jars containing damp cotton wool and the cages were surrounded with damp hessian.

A full description of the technique has been given in the second paper of this series.

In all, 2,892 specimens of 6 different species of mosquitoes were fed on the infected sheep, viz., 2,124 *A. caballus*, 639 *A. lineato-pennis*, 52 *A. hirsutus*, 50 *A. vittatus*, 24 *A. dentatus*, 3 *A. puncto-thoracis* and 2 *A. durbanensis*. More than double the number of specimens had to be caught and handled to obtain these results.

Scheme of Experiments.

The clean mosquitoes were fed on sheep experimentally infected with bluetongue virus during the actual temperature reaction. Later on, sheep infected or supposed to be infected by mosquitoes were also used. The mosquitoes were generally put on to the animals late in the afternoon and taken off again the following morning.

These infected mosquitoes were injected into susceptible sheep in the form of an emulsion in serum, or refed on susceptible sheep after an interval of at least 14 days, to allow for a certain development or multiplication of the virus to take place in the mosquito. Some of the mosquitoes were refed again after longer intervals.

The injections of crushed mosquitoes were mainly made as preliminary experiments to ascertain the presence or absence of the virus in some part of the body of the mosquito. To prove that a certain species is a transmitter, feeding experiments are naturally necessary. The exclusion of a species from the list of probable transmitters is more easily done by injection experiments.

When the experiment was concluded, every sheep was tested for immunity by means of subcutaneous injections of 1 or 2 c.c. bluetongue material from the same strain as that used for the infection of the mosquitoes. The immunity test was never applied earlier than 3 weeks after the feeding or injection of the mosquitoes in order to allow sufficient time for a reaction to appear.

First Series of Experiments.

We started our experiments with the ordinary *vaccine strain of bluetongue virus*. At first, in order to ascertain the presence of virus, the mosquitoes were injected into susceptible sheep after periods ranging between ½ and 19 days. In all, the following 12 experiments were made, in which about 250 mosquitoes, 124 *Aedes caballus*, 3 *A. dentatus*, 2 *A. hirsutus*, 112 *A. lineatopennis*, 1 *A. punctothoracis* and 9 *A. vittatus* were injected into 10 sheep.

Exp. 1.   5 *A. caballus* injected after ½ day.   Result positive.
Exp. 2.   20 *A. caballus* injected after 5 days.   Result doubtful.
Exp. 3.   30 *A. caballus* injected after 5 days.   Result negative.
Exp. 4.   40 *A. caballus* injected after 7 days.   Result negative.
Exp. 5.   14 *A. caballus* injected after 7 days.   Result negative.
Exp. 6.   15 *A. caballus* injected after 16-17 days.   Result negative.
Exp. 7.   20 *A. lineatopennis* injected after 5 days.   Result negative.
Exp. 8.   30 *A. lineatopennis* injected after 7 days.   Result negative.
Exp. 9.   2 *A. lineatopennis* injected after 16 days.   Result negative.
Exp. 10.   60 *A. lineatopennis* injected after 17-19 days.   Result positive.
Exp. 11.   6 *A. vittatus* injected after 5 days.   Result negative.
Exp. 12   ⎧ 3 *A. vittatus* injected after 7 days. ⎫
          ⎨ 3 *A. dentatus* injected after 7 days. ⎬ Result negative.
          ⎪ 2 *A. hirsutus* injected after 7 days. ⎪
          ⎩ 1 *A. punctothoracis* injected after 7 days. ⎭

In the first preliminary experiment 5 *Aedes caballus* were injected about 18 hours after they had fed on an infected sheep, with a positive result, showing that the 5 specimens had taken up sufficient virus to reproduce the disease. This result proved that the manner in which the mosquitoes were fed was efficient.

Negative results were obtained by injecting 99 *A. caballus* 5-17 days after they had fed on virus sheep; 52 *A. lineatopennis* after 5-16 days, 9 *A. vittatus* after 5-7 days, 3 *A. dentatus* after 7 days, 2 *A. hirsutus* after 7 days, and 1 *A. punctothoracis* after 7 days.

The injection of 30 *A. caballus* after 5 days was doubtful, the sheep showing a slight reaction not followed by immunity.

One experiment, in which 60 specimens were injected after 17-19 days was clearly positive, showing a typical fever reaction, complete immunity and clearly positive results with immunity in two subinoculations (experiment 10).

From these experiments we can conclude that the *bluetongue virus is capable of persisting in* Aedes lineatopennis *in a fully virulent form for periods of at least 17 days. This, however, was not regularly the case.* In 52 specimens of the same species no virus was present after 5-16 days.

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SECOND SERIES OF EXPERIMENTS.

In one of the experiments of the first series a doubtful reaction was obtained after an injection of *A. caballus*, the temperature suggesting a mild infection with bluetongue, which, however, showed no immunity when tested later. On this case a number of *A. dentatus*, *A. hirsutus* and *A. rittatus* were fed and the following 5 experiments carried out : —

Exp. 13.   9 *A. dentatus* injected after 5-6 days.   Result doubtful.
Exp. 14. 20 *A. hirsutus* injected after 5-6 days.   Result doubtful.
Exp. 15.   6 *A. hirsutus* injected after 16 days.   Result negative.
Exp. 16. 14 *A. rittatus* injected after 5 days.   Result negative.
Exp. 17.   8 *A. rittatus* injected after 20 days.   Result doubtful.

Two of these experiments (15 and 16) with 6 *A. hirsutus* injected after 16 and 14 *A. rittatus* after 5 days were certainly negative.

In the 3 other experiments (13, 14, 17) with specimens of *A. denatus*, *A. hirsutus* and *A. rittatus* injected after 5-20 days reactions resembling more or less the true bluetongue reactions were noticed after the injections of the mosquitoes, but no immunity followed or at any rate the immunity conferred was very weak. Subinoculations into 8 susceptible sheep failed.

In the last experiment (17), in which 8 *A. rittatus* were injected after an interval of 20 days the reaction that followed was, except for the short incubation period (4 days), indistinguishable from a true bluetongue reaction and was also accompanied by slight clinical symptoms. The immunity reaction was quite weak, suggesting that a certain degree of immunity had developed. However, as 4 sub-inoculations of blood taken during the fever period, failed, this experiment also has to be regarded as doubtful.

THIRD SERIES OF EXPERIMENTS.

Besides the doubtful case used for the second series a true case of bluetongue had been obtained in the first series of experiments by means of injections of 60 *A. lineatopennis* 17-19 days after their feeding. The virus had therefore undergone *one passage through mosquitoes*. On this sheep and two subinoculated animals 446 *Aedes caballus*, 136 *A. lineatopennis* and 10 *A. hirsutus* were fed, and with this material the following 9 experiments were carried out, in which this time the mosquitoes were refed on susceptible sheep after a period of at least 14-15 days had elapsed. Some of the mosquitoes were fed again after a longer interval or injected later into sheep as an emulsion.

Exp. 18. 16 *A. caballus* fed after 14-15 days.   Result negative.
Exp. 19. 25 *A. caballus* fed after 14-15 days.   Result doubtful.
Exp. 20. 10 *A. caballus* fed after 18-19 days.   Result doubtful.
Exp. 21.   1 *A. caballus* injected after 35-36 days.   Result negative.
Exp. 22.   8 *A. lineatopennis* fed after 14-20 days.   Result negative.
Exp. 23. 10 *A. lineatopennis* injected after 22 days.   Result doubtful.
Exp. 24. 15 *A. lineatopennis* fed after 14-15 days.   Result positive.
Exp. 25. 16 *A. lineatopennis* fed after 18-26 days.   Result doubtful.
Exp. 26.   3 *A. hirsutus* fed after 17 days.   Result doubtful.

One of the experiments (No. 24) was regarded as *positive*. In this case 15 specimens fed after an interval of 14-15 days. 12 days later a temperature reaction, however, not very marked, occurred. When tested 18 days later for immunity, the animal proved to be normally susceptible. Blood taken during the short febrile reaction, was injected into two sheep. One of these animals developed a marked fever reaction, accompanied by slight but typical clinical symptoms, and when tested for immunity it proved to be totally immune. Virus must, therefore, have been present in the original mosquito infected sheep, although no immunity was conferred to it.

Clearly negative results were obtained in only 3 experiments, in which 16 *A. caballus* were fed after 14-15 days, 8 *A. lineatopennis* after 14-20 days, and in which 1 *A. caballus* was injected after 35-36 days.

In the remaining 5 experiments the results were doubtful. In these experiments 35 *A. caballus* had been fed after 14-19, 16 *A. lineatopennis* after 18-26, and 3 *A. hirsutus* after 17 days, whereas 10 *A. lineatopennis* were injected after 22 days. In one of the experiments with *A. caballus* a short reaction occurred after the feeding of the mosquitoes, followed later by an immunity reaction of a similar intensity. In the second experiment a fairly typical reaction commenced 11 days after the feeding of the mosquitoes but it did not confer any immunity to the sheep. In both cases sub-inoculations into susceptible sheep failed to give a positive result. In the experiment in which 10 *A. lineatopennis* were injected, no definite reaction followed the injection, but the result of the immunity test was not reliable. In the other experiment in which specimens of the same species were fed after 16-18 days, quite a typical temperature reaction occurred, but it was not followed by immunity and subinoculations of blood into susceptible sheep were negative. In the experiment with *A. hirsutus* the result of the immunity test was very doubtful. The animals used in these experiments normally showed a regular temperature.

Fourth Series of Experiments.

Three of the sheep of the preceding series of experiments were used for feeding further mosquitoes. Two of them had shown temperatures more or less closely resembling bluetongue reactions, but not followed by immunity after the feeding of *A. caballus* or *A. lineatopennis*, and the third animal had had a true bluetongue reaction after being injected with blood from another sheep. The vaccine strain had thus passed twice, truly or supposedly, through mosquitoes.

On these three sheep 382 *A. caballus* and 75 *A. lineatopennis* were fed and they were refed in the following 6 experiments after intervals ranging between 14 and 20 days.

Exp. 27. 2 *A. caballus* fed after 15 days. Result negative.
Exp. 28. 41 *A. caballus* fed after 15 days. Result doubtful.
Exp. 29. 36 *A. caballus* fed after 21 days. Result negative.
Exp. 30. 40 *A. caballus* fed after 14 days. Result doubtful.
Exp. 31. 17 *A. caballus* fed after 20 days. Result negative.
Exp. 32. 10 *A. lineatopennis* fed after 15 days. Result negative.

None of these experiments were positive.

Clearly negative results were obtained in 3 experiments, in which 55 *A. caballus* fed after 15-21 days, and 10 *A. lineatopennis* after 15 days.

Doubtful results were obtained in 2 experiments, in which respectively 41 and 40 *A. caballus* fed after 15 and 14 days. In one of these experiments a temperature reaction set in just prior to the immunity test and continuing through its incubation period, whereas in the other experiment just before the application of the immunity test, which was positive, another reaction of equal strength had occurred.

FIFTH SERIES OF EXPERIMENTS.

At the beginning of April, 1932, material from a natural case of bluetongue was received from *Lxopo*. Blood from this case injected at Onderstepoort into 2 sheep gave only weak reactions. On these sheep 413 *A. caballus*, 10 *A. lineatopennis* and a few *A. durbanensis* were fed. At that time of the year *A. caballus* was the only species obtainable in fair numbers. The mosquitoes were refed in the following experiments after intervals ranging from 15 to 29 days and those remaining alive after 1½ months were injected into sheep.

Exp. 33.   13 *A. caballus* fed after 15 days. Result negative.
Exp. 34.   14 *A. caballus* fed after 15 days. Result negative.
Exp. 35.   4 *A. caballus* fed after 23-24 days. Result negative.
Exp. 36.   2 *A. caballus* injected after 45-46 days. Result negative.
Exp. 37.   6 *A. lineatopennis* fed after 15-29 days. Result negative.
Exp. 38.   2 *A. lineatopennis* injected after 45 days. Result negative.
Exp. 39.   1 *A. durbanensis* fed after 16 days. Result negative.

In all, 31 *A. caballus* were fed after 15-24 days and 2 were injected after 45-46 days, 6 *A. lineatopennis* (of which 2 were doubtful) fed after 15-29 days, 2 were injected after 45 days and 1 *A. durbanensis* fed after 16 days. All the experiments were negative, whereas the sheep used proved to be normally susceptible.

SIXTH SERIES OF EXPERIMENTS.

At the beginning of April, 1932, we received material from another natural case, which had occurred at the farm Kameelfontein in the Pretoria District. Injected at Onderstepoort into sheep, it gave temperature reactions of medium to marked intensity and slight clinical symptoms in one of the further subinoculations.

On three virus sheep 402 *A. caballus* and 12 *A. lineatopennis* could be fed and they were refed in the following experiments after intervals lasting at least 14 days.

Exp. 40.   15 *A. caballus* fed after 15 days.   Result negative.
Exp. 41.   4 *A. caballus* fed after 25 days.   Result negative.
Exp. 42.   31 *A. caballus* fed after 15 days.   Result negative.
Exp. 43.   9 *A. caballus* fed after 21 days.   Result negative.
Exp. 44.   4 *A. caballus* fed after 15-20 days.   Result negative.
Exp. 45.   6 *A. lineatopennis* fed after 14-21 days.   Result negative.
Exp. 46.   1 *A. lineatopennis* injected after 38 days.   Result negative.

In these 7 experiments 63 *A. caballus* were refed on sheep after intervals ranging between 15 and 21 days, whereas finally 1 *A. lineatopennis* was injected after 38 days. All the experiments were negative and the 7 sheep used proved later to be normally susceptible to the Kameelfontein strain.

General Discussion of the Results.

In all, 46 experiments were made with three different strains of bluetongue, a vaccine strain and two others derived from fresh spontaneous cases.

In these experiments 324 specimens were injected after periods ranging between $\frac{1}{2}$ and 45 days and infected mosquitoes were refed 346 times after 14-29 days, viz. : —

     A. caballus, 127 injected, 281 refed.
     A. lineatopennis, 125 injected, 61 refed.
     A. vittatus, 31 injected.
     A. hirsutus, 28 injected, 3 refed.
     A. dentatus, 12 injected.
     A. punctothoracis, 1 injected.
     A. durbanensis, 1 refed.

Three positive results were obtained. In the first case some *Aedes caballus* were injected the day after their feeding and thus the result has no relation to the actual capacity for transmission. In the second experiment a positive infection was obtained by the injection of 60 *Aedes lineatopennis* 17-19 days after their feeding on a virus sheep and this infection could be transmitted further through subinoculations. The third case was not absolutely clear cut. 15 *A. lineatopennis* had fed 14-15 days after their original infection, the feeding being followed by a slight temperature reaction, but not by immunity. Through subinoculations, however, the presence of virus could be definitely traced during the febrile reaction.

The results were doubtful in 10 experiments in which 30 *A. caballus*, 10 *A. lineatopennis*, 20 *A. hirsutus*, 8 *A. vittatus* and 9. *A. dentatus* were injected after 5-22 days and 116 *A. caballus* fed after 14-26 days. In these experiments temperature reactions, not followed by immunity and not confirmed by subinoculations, were observed, or the result of the immunity test was open to doubt.

32 Experiments were negative, the vast majority, in which 182 mosquitoes were injected and 199 specimens refed. 92 *A. caballus*, 55 *A. lineatopennis*, 23 *A. vittatus*, 8 *A. hirsutus*, 3 *A. dentatus* and 1 *A. punctothoracis* were injected after intervals of 5-46 days, whereas 165 *A. caballus*, 30 *A. lineatopennis*, 3 *A. hirsutus* and 1 *A. durbanensis* were refed after 14-29 days. In 5 of these experiments, in which 14 *A. vittatus* and 6 *A. hirsutus* were injected and 38 *A. caballus* and 10 *A. lineatopennis* refed, the mosquitoes had been originally fed on cases in which there could be some doubt as to the true nature of the temperature reaction.

As negative experiments, of which there is no doubt, there remain 27, in which 162 specimens, 92 *A. caballus*, 55 *A. lineatopennis*, 9 *A. vittatus*, 2 *A. dentatus*, 2 *A. hirsutus* and 1 *A. punctothoracis* were injected and 151 specimens, 127 *A. caballus*, 20 *A. lineatopennis* were refed after the same intervals as stated above.

Only with *A. caballus* and *A. lineatopennis* could experiments with large numbers be made.

Both positive experiments were obtained with *Aedes lineatopennis*. Against one positive case obtained by injection of 60 specimens after 17-19 days, there are negative results with 50 specimens injected after 5-7 and 5 after 16-45 days, and against the positive result obtained through feeding stand 3 negative experiments, in which 20 specimens were refed after 14-29 days.

All the experiments with *A. caballus* were negative. 92 specimens were injected, 74 after 5-7 days, 15 after 16-17, and 3 after 35-46 days. 127 specimens were refed, 92 after 14-15 days and 35 after 20-25 days. The material used was large enough to allow of a definite conclusion being arrived at.

Some facts may have influenced the results of these experiments. The vaccine strain, with which most of the work was done, was not a very suitable one. It had been isolated about 5 years previously and passed through nearly 50 generations by means of direct inoculations. Through these direct inoculations it had been profoundly altered, attenuated, and it is possible that at the same time its developmental capacity in insects had also been changed, viz., reduced. The Ixopo strain, though recently isolated from a natural case, gave very slight reactions, suggesting that during the temperature reactions the amount of virus present in the blood was not very large. It may further be possible that only a certain period in the duration of the febrile reaction is suitable for the infection of the insects.

In the interpretation of the results we were as careful as possible, and some of those regarded as doubtful may actually have been positive.

Arriving at the final conclusions we may say:—

Aedes lineatopennis seems *to be a natural transmitter of bluetongue*. Our results do not allow us to state whether this species is an important transmitter or only a more or less accidental carrier, as the number of positive results was too small.

Unfortunately, when we did obtain material from natural cases towards the end of the season this species was only available in very small numbers.

From an epizootological point of view, *Aedes lineatopennis* would be a very suitable transmitter. Breeding only in temporary water, it depends absolutely on the amount of rain. It breeds during the summer after good rains in a large number of marshy

spots in the veld, provided thay are covered with grass. After the mosquitoes have hatched out, the adults remain for some time at or near the breeding places, which usually lie in vleis. The breeding habits and behaviour of the adults is just what one would expect of an efficient transmitter.

Aedes caballus does not seem to be a transmitter at all. The number of negative experiments was sufficient to warrant this conclusion.

The material used of other species is too small to allow of any definite conclusion. However, according to the negative results, although small in numbers, it is improbable that they are very important carriers.

In future work the real importance of *A. lineatopennis* will first of all have to be decided, and if this should happen not to be in favour of this species, more work with the *Stegomyia* group and with Anophelines will have to be carried out. The possibility still remains that Arthropoda other than mosquitoes may be involved in the transmission of this disease.

SUMMARY.

During the summer 1931/32 transmission experiments with bluetongue in sheep were carried out at Onderstepoort. Owing to lack of rain, the season was very unfavourable for our work.

The result of a mosquito survey had pointed to *Aedes* species as being very suitable transmitters from an epizootological point of view, and some species of this genus were used for our experiments.

Three strains of virus were used, the vaccine strain of the laboratory and 2 strains from natural cases, obtained towards the end of the season.

Nearly 3,000 clean mosquitoes were fed on 18 infected sheep, the majority belonging to *Aedes caballus* and *A. lineatopennis*, and only relatively small numbers of 5 other species including *A. hirsutus*, *A. vittatus* and *A. dentatus*.

In 22 experiments, 324 specimens were injected into sheep after periods ranging between $\frac{1}{2}$ and 45 days, 127 *A. caballus*, 125 *A. lineatopennis*, the remainder belonging to *A. vittatus*, *A. hirsutus*, *A. dentatus* and *A. punctothoracis*.

Infected mosquitoes were refer 346 times after 14-29 days on susceptible sheep at periods varying between 14 and 29 days, viz., 281 *A. caballus*, 61 *A. lineatopennis* and 4 *A. hirsutus* and *A. durbanensis*.

Three positive results were obtained. In the first case 5 mosquitoes were injected shortly after their infection, showing only that sufficient virus was taken up. In the second case 60 *A. lineatopennis*

were injected after 17-19 days, and in the last experiment 15 specimens of the same species refed after 14-15 days. In the last case no immunity was acquired but the presence of virus could be ascertained by subinoculation.

All the other experiments were either negative or the results doubtful.

Aedes lineatopennis seems to be a transmitter of bluetongue, very adapted for this purpose from an epizootological point of view owing to its breeding habits and behaviour in the adult stage. It could not be ascertained if this species is an important or only a more or less accidental transmitter.

Aedes caballus does not seem capable of transmitting the disease.

The work carried out up to now does not present a solution of the problem of the natural transmission of bluetongue.

Section II.

Mineral Metabolism and Deficiency.

Studies in Mineral Metabolism XXXI.

Minimum Mineral Requirements of Cattle. (2nd REPORT).*

By

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I. INTRODUCTION.

INCREASED production of milk and beef, like the improvement of herds, is closely associated with good feeding, while improved nutrition can only be brought about if feeds in all their constituents satisfy the requirements of the animals. Minerals, as is probably universally realized in both agricultural research and practice, play an important part in the rations of cattle kept for high production. Furthermore, the feeding of mineral constituents to stock has certainly had at least its fair share of attention during the last dozen years and, like most matters that are suddenly projected into the limelight, has been stressed and emphasized almost to the exclusion of other equally important problems. Perhaps a reason for this decided emphasis of the mineral composition of the diet has been the belief that a lactating cow assimilates probably only about 20 per cent. of the minerals present in her feed, and that consequently milk production is a heavy drain upon the animal's mineral reserves. Then too, the phenomenal improvement in the growth rate of cattle on supplying the naturally deficient mineral, especially phosphorus in certain countries, has given impetus to the study of the mineral constituents of the diet, with the result that while a balanced ration was assumed to contain sufficient minerals for the requirements of stock in the past, in recent years the practice has become prevalent to supply additional minerals to those contained in the ration of dairy cows and even of non-lactating grazing animals.

II. OBJECT OF THE INVESTIGATION.

It was with the view to determine the minimum mineral requirements of growing cattle that a study of this problem was begun in 1925. The first report of the work was published in 1927 while the experiments recorded in this article form a continuation and in some ways an extension of the original object of studying the mineral requirements of growing heifers. As in the first investigation, a pair of Grade Friesland heifers, 30 months old, was placed in each experiment, which was continued through the growing period and until the end of the first three months of the second lactation period. Full details of each experiment and its results are given in the text below.

III. LITERATURE.

No attempt will be made to review recent literature on mineral metabolism, since this subject has recently been discussed by Crichton (1930), who gives a fairly extensive list of references, and although one regrets that the earlier work of investigators like Marek, Wellman and Sjollema in the field of mineral metabolism has been omitted, the review of the literature is undoubtedly a masterly one. Calcium and phosphorus predominate in all the work mentioned. As a matter of fact Crichton deplores the absence of data on the rôle of other inorganic constituents such as magnesium, sodium, potassium and chlorine in animal nutrition, while he states that knowledge of the mineral requirements of growing cattle is regrettably scanty.

The position as set forth by Crichton with regard to the mineral requirement of dairy cattle may be summed up as follows: The literature provides abundant proof for the limitation set upon growth and milk production by deficient phosphorus in the diet under natural conditions of grazing. Under experimental conditions clinical symptoms of aphosphorosis may develop. With regard to calcium the position is not so clear cut. There is no direct evidence that pastures exist that are so deficient in calcium that the growth rate or health of stock is affected, but the author doubts whether such cases do not occur. Calcium deficient diets have given positive results under experimental conditions. Concerning other minerals present in milk, there is almost no information as to the requirements of cattle and "there is no information on which to base even an opinion as to whether or not deficiency of some of these may affect milk yield or health". Chlorine is liable to be deficient, but this is easily made good by providing rock or ordinary salt. Crichton concludes by expressing the view that the grading up of pastures, although a slow business to ensure adequate mineral intake must be given preference to the addition of inorganic salts to deficient rations.

On the whole we are in agreement with the latter view as an ideal and grading up is comparatively simple where artificial pastures are concerned, but it does not as yet, except in a very small way, fall within the scope of practical agricultural politics where natural, mixed pastures are concerned. It has not yet been shown that phosphorus deficiency, undoubtedly the most widespread mineral deficiency in natural pastures can be made good in this way, so that supplementary feeding of minerals to stock is still an important method of overcoming mineral deficiencies in the natural feed of animals.

An aspect of the cause of phosphorus deficiency in natural pastures which is often overlooked and which throws some light on the puzzle of an existing phosphorus deficiency in practically all sub-tropical areas with seasonal rainfalls not well distributed over the year and hence subject to droughts, lies in the variation of the phosphorus content of pastures with growth. Generally, young succulent grass high in phosphorus is available only for a comparatively short period, while full-grown, mature grass, low in phosphorus, forms the staple food for perhaps eight of the twelve months even in areas where the soil is not necessarily deficient in phosphorus. Values of ·75 per cent. P_2O_5 for young grass may change quite normally to ·03 per cent. for the same grass when mature. Unless pasture improvement develops a short cut to the establishment of pastures consisting mainly of grasses which do not show a remarkable drop in their phosphorus content on maturing, supplementary phosphorus feeding in pastoral countries like South Africa and parts of Australia, will of necessity still be practised for a long time.

Reed and Huffman (1930) report on a five-year mineral feeding investigation with dairy cattle and state that the mineral requirements of dairy cows under conditions of normal production and stall feeding of balanced rations are probably satisfied without the addition of supplementary minerals.

Marek (1924), Wellman (1931) and Marek and Wellman (1932) place great stress upon ratios of minerals to one another which is implied in their conception of " Alkali-alkalizität " and " Erdalkali-alkalizität ". These authors explain rickets and allied conditions along lines of abnormal Erdalkali-alkalizität ($MgO + CaO - P_2O_5$ in milligram equivalents per 100 grams dry matter of the ration). A well balanced and healthy ration should show a value between 20 and 25 mgm. equivalents and it is interesting to note that many so-called well balanced rations will not be approved of by these two authors or may even be labelled as definitely rachitic. A vast amount of data obtained mainly from experiments with pigs during a score of years, is presented in two volumes under joint authorship and although much is stated which is not in agreement with the current conception of mineral metabolism, the work certainly demands the serious attention of investigators in this field of research.

IV. DETAILS OF INVESTIGATION.

In the experiments recorded in this paper an attempt was made to subject heifers to extremely low intakes of mineral constituents and, as in the 1925 experiment, roughage was reduced to a minimum in order to obtain a basal ration sufficiently low in all minerals to be satisfactory for all the groups, and so place them on a common basis for comparison as far as food consumption is concerned. It was found that $3\frac{1}{2}$ lb. hay, 5 lb. crushed maize and 5 lb. maize endosperm or fanko, gave a basal ration low in mineral but adequate in other respects in that it produced normal growth if the minerals required were given as a supplement. The total intake of minerals and the mineral composition of the ration are given in Table I.

TABLE I.
Percentage Mineral Composition of Materials used.

Material.	CaO.	MgO.	K_2O.	Na_2O.	P_2O_5.	Cl.	SO_3.	Protein.
Hay.........	·35	·25	1·2	·08	·13	·25	·40	4·8
Maize.......	·03	·24	·36	·16	·5	·08	·16	9·0
Fanko.......	·01	·05	·14	·02	·09	·03	·14	8·0
Blood meal..	·2	·03	·40	1·6	·60	1·0	·8	64·8
Ensilage.....	·10	·07	·3	·01	·1	·06	·1	1·6
Meat meal...	1·1	·04	·4	1·4	1·4	·85	·7	80·0

The initial basal ration of 3·5 lb. hay, 5 lb. crushed maize, 5 lb. fanko, and 20 gm. blood meal contained the following approximate amounts of constituents in grams : —

Constituent ... ... CaO MgO K_2O Na_2O P_2O_5 Cl SO_3 Protein.
gm. per head per day 6·8 11·1 29·3 5·7 15·4 6·7 12·9 472

Due to the high cost of fanko the initial plan of omitting maize and increasing the fanko to 10 lb. had to be abandoned. Maize is considerably higher than fanko in minerals, especially phosphorus; so much so that it was impossible to produce an acute phosphorus deficiency on the above ration, and fanko had to be substituted for maize in the phosphorus deficient groups as indicated further in this

article. Blood meal was added to improve the " quality " of the protein of which the ration contained sufficient for growing heifers weighing approximately 800 lb. on an average.

The experiment was begun in September, 1930. The animals were stabled under roof in separate feeding boxes over night in a shed. The fanko, maize and blood meal were fed in the afternoon at 3 o'clock when the animals were fastened to their mangers. In the early morning the hay was given which was invariably consumed before half-past 9 when the animals were allowed to go into a fair sized paddock with concrete floor for exercise until stabling time in the afternoon. Water was always available and the minerals were added to the concentrates of the ration. Each animal was placed on the experimental ration immediately after service.

Twenty-six grade Friesland heifers, 2½ years old, were available and pairs, as uniform as possible, were placed in each experiment. With the exception of one, all the animals calved in May and June, 1931. Each was milked for a 90-day period then allowed to dry off and placed with the same bull for a second service in December, 1931.

In February, 1932, 5 lb. maize ensilage was added daily to the basal ration which, therefore, altered the mineral intake per head to the following values: $CaO = 8 \cdot 8$ gm., $MgO = 12 \cdot 5$ gm., $K_2O - 35 \cdot 3$ gm., $Na_2O - 5 \cdot 9$ gm., $P_2O_5 = 17 \cdot 4$ gm., $Cl = 8 \cdot 0$ gm., $SO_3 = 14 \cdot 9$ gm. The protein content of the new basal ration was 510 gm. At the beginning of the second lactation period additional protein was given in the form of $1 \cdot 5$ lb. of meat meal. A summary of the intake of minerals contained in the basal ration over the whole experimental period is given below in grams:—

Period.	CaO.	MgO.	K₂O.	Na₂O.	P₂O₅.	Cl.	SO₃.	Protein.
±17.9.30– 18.5.32...	6·8	11·1	29·3	5·7	15·4	6·7	12·9	472
18.5.32– 8.8.32....	8·8	12·5	35·3	5·9	17·4	8·0	14·9	510
Last 3 months	15·1	12·8	38·0	15·5	27·0	13·8	19·5	1,026

Prior to the experimental period the roughage of the ration was gradually reduced to that contained in the basal ration. During the preliminary period beginning on 11.5.30 each animal was put on a ration consisting of 3½ lb. lucerne hay, 5 lb. maize, 5 lb. fanko, 20 gm. blood meal, 4 ounces bone meal, until gestation began when the basal ration given for the period 17.9.30–18.5.32 was given instead.

Bearing in mind the basal rations for the periods given, the arrangement of the heifers into twelve groups of one pair each will be clear. An attempt was made to keep the total intake of minerals in all the groups constant except the one constituent which was kept low intentionally. The quantities of mineral supplements given were based upon the composition of 12 lb. good English hay and an

attempt was made to approximate the quantity of minerals in the ration and supplement to that which would be ingested by heifers of this type on English pasture. The following table contains a schematic representation of the experiment and indicates at the same time the intake of minerals in the various groups.

TABLE II.

Daily intake of Minerals per head in Basal Ration plus Mineral Supplement.

D.O.B. Nos.	Experiment.	Period.	CaO.	MgO.	K₂O.	Na₂O.	P₂O₅.	Ce.	SO₃.
	I.								
3641	Low Ca and low P......	19.7.30 to 17.2.32	6·8	21·4	76·3	18·9	15·4	57·4	12·9
3648		18.2.32 to 17.5.32	6·3	16·3	72·3	16·7	8·4	56·2	12·9
		18.5.32 to 8.8.32	8·3	17·3	78·3	16·9	8·4	57·5	14·9
		Last three months	14·8	18·0	81·0	27·5	17·4	63·3	19·5
	II.								
3659	Low P...............	19.7.30 to 17.2.32	51·6	21·4	76·3	18·9	15·4	57·4	12·9
		18.2.32 to 17.5.32	51·1	16·4	72·3	15·8	6·4	56·2	12·9
		18.5.32 to 8.8.32	53·1	17·8	80·3	16·0	8·4	57·5	14·9
		Last three months	59·1	20·1	83·0	25·6	18·0	65·3	19·5
	IIIᴀ.								
3643	Low Ca.......	19.7.30 to 17.5.32	6·8	21·4	74·0	18·9	45·1	49·8	12·9
3655		18.5.32 to 8.8.32	8·8	22·8	80·0	19·1	46·8	50·2	14·9
		Last three months	15·1	23·1	80·0	28·7	56·7	56·0	19·5
	IIIʙ.								
3640	Low Ca and low Mg.....	19.7.30 to 17.5.32	6·8	11·1	101·3	18·9	45·1	48·9	12·9
3650		18.5.32 to 8.8.32	8·8	12·5	107·3	19·1	46·8	50·2	14·9
		Last three months	15·1	12·8	110·0	28·7	56·7	56·0	19·5
	IV.								
3642	All mineral deficiency except P. and Ca	19·7·30 to 17.5.32	47·1	11·1	29·3	5·7	45·1	6·7	12·9
3649		18.5.32 to 8.8.32	49·1	12·5	35·3	5·9	46·8	8·0	14·9
		Last three months	55·4	12·8	38·0	15·5	56·7	13·8	19·5

TABLE II—(continued).

D.O.B. Nos.	Experiment.	Period.	CaO.	MgO.	K$_2$O.	Na$_2$O.	P$_2$O$_5$.	Cc.	SO$_3$.
	V.								
3651	Low Na and low Cl. ...	19.7.30 to 17.5.32	56·1	21·4	75·0	5·7	45·1	6·7	12·9
3646		18.5.32 to 8.8.32	53·6	22·8	80·0	5·9	46·8	8·0	14·9
		Last three months	59·9	23·1	80·0	15·5	56·7	13·8	19·5
	VI.								
3658	Low Cl................	19.7.30 to 8.8.32	51·6	21·4	76·3	18·9	45·1	6·7	12·9
3675		18.5.32 to 8.8.32	53·6	22·8	80·0	19·1	46·8	8·0	14·9
		Last three months	59·9	23 1	80·0	28·7	56·7	13·8	19·5
	VII.								
3653	Low Na................	19.7.30 to 17.5.32	51·6	21·4	76·3	5·7	45·1	57·4	12·9
		18.5.32 to 8.8.32	53·6	22·8	82·3	5·9	46·8	58·7	14·9
		Last three months	59·9	23·1	80·0	15·5	56·7	64·5	19·5
	VIII.								
3673	Low K................	19.7.30 to 17.5.32	51·6	21·4	29·3	18·9	45·1	57·4	12·9
3656		18.5.32 to 8.8.32	53·6	22·8	35·3	19·1	46·8	46·8	14·9
		Last three months	59·6	23·1	38·0	28·7	56·7	53·8	19·5
	IX.								
3677	All mineral sufficiency, plus Kl	19.7 30 to 17.5.32	47·1	21·4	76·3	18·9	45·1	57·4	12·9
3652		18.5.32 to 8.8.32	49·1	22·8	82·3	19·1	46·8	57·4	14·9
		Last three months	55·4	23·1	85·0	28·7	56·7	64·5	19·5
	X.								
3645	All mineral sufficiency ..	19.7.30 to 17.5.32	47·1	21·4	76·3	18·9	45·1	57·4	12·9
		18.5.32 to 8.8.32	49·1	22·8	82·3	19·1	46·8	58·7	14·9
		Last three months	55·4	23·1	85·0	28·7	56·7	64·5	19·5

It will be seen from Table II that eleven groups each comprising a pair of heifers were formed. Originally there were 12 pairs but the group receiving a supplement of sodium fluorine soon showed

clinical symptoms of fluorine poisoning as reported in an article by Du Toit and others (1932) and was perforce eliminated when the animals died, about 12 months after the beginning of the experiment. On the whole the mineral intake remained constant for all groups except, of course, that the constituent intended to be low in the ration of a particular group is restricted to the amount contained in the basal ration only. For instance, the phosphorus intake of animal No. 3659 viz. 15·4 gm. in Experiment II is intentionally different from that of the animals in Experiment IV, but the amount of other minerals contained in these two rations is the same for both groups.

In Experiments I and II the basal ration containing 5 lb. fanko and 5 lb. maize was changed on 18.2.32 to 10 lb. fanko or maize endosperm and no maize, thereby decreasing the phosphorus intake very considerably (from 15·4 to 6·4 gm.), without altering the rest of the composition of the ration markedly.

In the following pages each of the groups or experiments set forth in Table II will be considered separately and comparisons made with the control animal in Experiment X, the ration of which was supplemented to be sufficient in all the minerals studied. Unfortunately animal 3639, the second control heifer, was injured and had to be eliminated from the experiment. One of the heifers in Experiment II died of Heartwater (rickettsiosis) shortly after the beginning of the experiment and left only one in that group. It is unfortunate that only one control heifer remained in this investigation, but if a comparison is made of the growth curve of this heifer, viz. No. 3645 with that of the control group kept under similar conditions in the experiments described by Theiler, Green and Du Toit (1927) it will be seen that this heifer can well be regarded as the standard of comparison in the present investigation. Her increase in weight, general health, food consumption, etc., agree well with those of the controls in the original experiment.

Reference to the weight curves will make the basis of comparison of the groups clear. Naturally the animals in the different groups did not calve on the same date, yet it is obviously necessary, if comparisons are to be made, to compare a lactating animal of a group with its mate during the same period of lactation. The same applies when groups are compared. Hence the two successive dates of calving of all the animals were made to coincide. The two periods of lactation and of gestation will then obviously coincide. The length of the rest periods were different and these are indicated in figures on each curve. Direct comparisons between two animals are, therefore, made for the respective gestation and lactation periods in the graphic representations submitted below.

The animals were weighed at monthly intervals, when samples of blood were drawn for analysis. Food consumption was registered from time to time as stated in the discussions of the individual experiments. Milk production was registered and monthly milk samples analysed for minerals as well as for the organic constituents. This aspect of the work will be reported on in detail by one of the authors (J. W. G.) at a later date.

The basal ration of 3·5 lb. hay, 5 lb. fanko, 5 lb. maize, 20 gm. blood meal was given daily for the first 20 months of the experiment,

when for reasons explained in the general discussion of the work, 5 lb. of maize ensilage was given in addition. Actually, therefore, the animals were not receiving enough protein for milk production during the first lactation period, while during second lactation protein deficiency was rectified by the daily addition of 1·5 lb. meat meal (80 per cent. protein.)

The basal rations for the three periods of the investigation were as follows:—

Period.	Daily Basal Ration.
1. Beginning of first gestation (Sept., 1930)–17.5.32	3·5 lb. hay, 5 lb. crushed maize, 5 lb. fanko, 20 gm. blood meal.
2. 18.5.32–8.6.32............	Basal ration of 1st period, plus 5 lb. maize ensilage.
3. Last 3 months of investigation	Basal ration of 2nd period, plus 1·5 lb. meat meal (80 per cent. protein).

Reference to Table II reveals 3 corresponding periods of mineral intake as suggested by the above, except in Experiment I which has been explained elsewhere. Food consumption was registered daily and the animals inspected for clinical symptoms of disease or of deficiencies.

EXPERIMENT I.

Low calcium and low phosphorus but adequate in other respects.

History.

No. of animal....................	No. 3641	No. 3648	No. 3645 (control.)
Date when experimental ration began, i.e. beginning of 1st gestation......	28.8.30	1.9.30	29.10.30
Date of calving..................	4.6.31	3.6.31	19.7.31
End of lactation.................	4.9.31	3.9.31	19.10.31
End of rest period, i.e. beginning of 2nd gestation......................	15.11.31	5.3.32	14.12.31
Date of calving..................	26.8.32	12.12.32	25.9.32
Date of conclusion of experiment......	23.9.32 (killed *in extremis*.)	12.3.33	25.12.32

The basal ration of 3½ lb. hay, 5 lb. crushed maize, 5 lb. fanko, and 20 gm. blood meal was given, plus 25 gm. NaCl, 15 gm. $Mg(OH)_2$ and 75 gm. KCl. The mineral constituents in the day's food came to 6·8 gm. CaO, 15·4 gm. P_2O_5, 21·4 gm. MgO, 76·3 gm. K_2O, 18·9 Na_2O, 57·4 gm. Cl and 12·9 gm. SO_3. Except for phosphorus and calcium the mineral content of this ration was the same as that given to the control animal No. 3645 on a ration sufficient in all minerals. The ration is potentially alkaline with a positive Erdalkali-alkalizität of approximately 24 milligram equivalents and a ratio of CaO to P_2O_5 of 1:2·2.

It should be stated, however, that this experiment was conceived with the intention of producing deficiencies in phosphorus and calcium apart from the fact whether a favourable or unfavourable calcium phosphorus ratio, or Erdalkali-alkalizität was present or not. These factors may and probably do influence the severity of a deficiency, and

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its effect upon the system; but if an absolute deficiency can be brought about, factors influencing its effect upon the organism or its acuteness must be of secondary importance. That was the attitude towards such factors at the beginning of the investigation and hence they were left out of consideration, although it was realized that they would probably not be without effect. This course was adopted in the belief that the deficiency would be sufficiently acute in any case to be recognized in spite of favourable factors.

In an experiment now being undertaken calcium and phosphorus metabolism is being studied on a broader basis with due regard to the effect upon the absorption, retention and utilization of these two constituents, of modifying factors such as varying ratios and changes in the Erdalkali-alkalizität in Marek's sense.

The comparative weight increases of animals 3641 and 3648 in Experiment I are given in Graph I. The weight curve of the control animal 3645 is given on the same chart for purposes of comparison. A graphic representation of the inorganic phosphorus and of calcium in the blood of these three animals is given in Graph II.

Apparently both animals stood the strain of their first pregnancy well. During lactation the heavier producer 3641 lost more weight, showed hardly any improvement during her comparatively short rest period of 72 days, and withstood the strain of gestation until about 2 months before calving, when she began to lose weight rapidly, and it became obvious that she would not live much longer. She calved normally when in poor condition, continued losing weight,
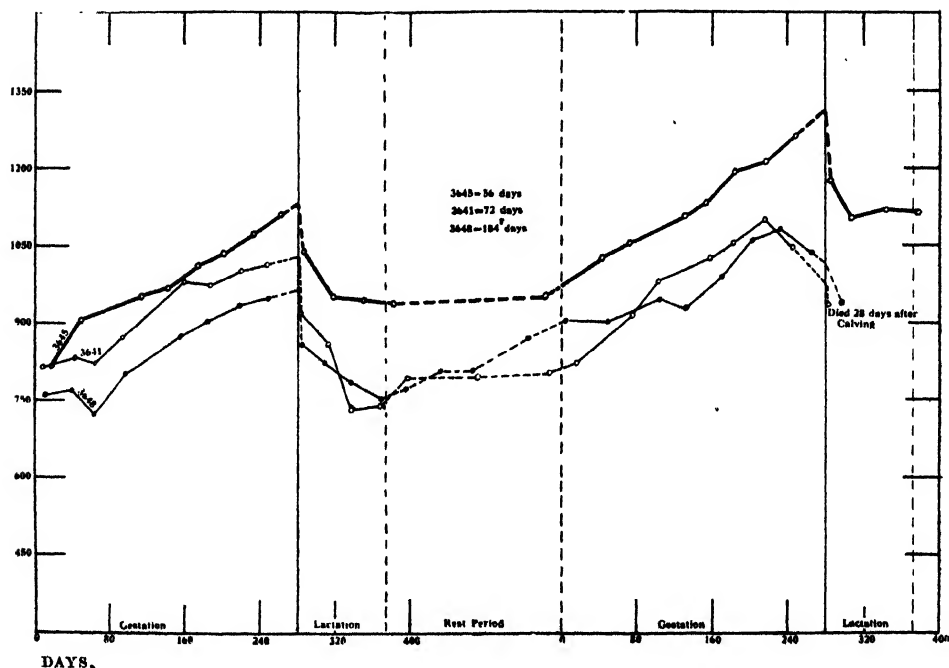
GRAPH I.—Weights of Animals in Pounds.
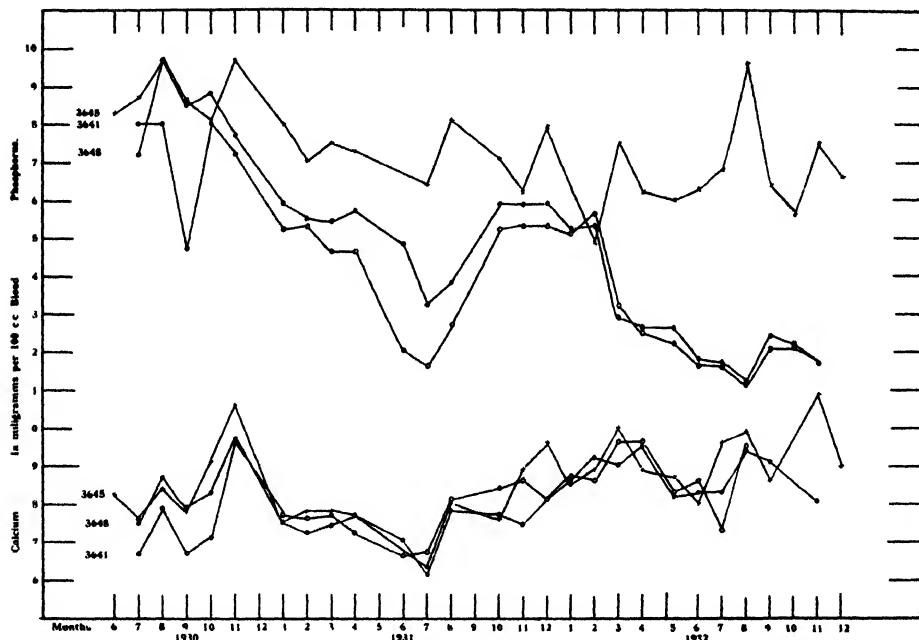
GRAPH II.—Inorganic Phosphorus and Calcium.

and when about a month after calving she was unable to rise and in a miserable state, she was killed in extremis. No. 3648 fared slightly better. She was a lighter milk producer and had a comparatively long rest period of 184 days during which she showed great improvement. In spite of her better chances this animal also began to lose weight before calving, was reduced to very poor condition during lactation, could hardly walk on account of acute styfsiekte (aphosphorosis), and died of shock following dislocation of the hip in a fall, 10 days before the end of lactation.

Both animals did poorly when compared with the control and developed clinically recognizable stywesiekte, early in the experiment. This condition lasted throughout with improvement during the rest period when the phosphorus intake was most probably not far short of the requirements of the animals. The amount of inorganic blood phosphorus confirms this view. It rose after the first lactation to a normal figure of over 5 mgm. per 100 c.c. until the 5 lb. maize in the basal ration were substituted by 5 lb. fanko in February, 1932. After that period the inorganic phosphorus dropped indicating aphosphorosis which lasted until the end of the experiment. Furthermore, Graph II also indicates that the animals in Experiment I most probably did not feel the effects of the low phosphorus in the ration before the onset of first lactation.

The values for calcium in the blood of these animals give no indication as to whether a calcium deficiency existed.

For a comparison of the intake and outgo of calcium and phosphorus in the food on the one hand and in the milk on the other, reference must be made to Tables III and IV.

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TABLE III.
Daily Mineral Intake in Feed and Output in Milk for First Lactation.

Group	D.O.B.	CaO Intake	CaO Outgo	P₂O₅ Intake	P₂O₅ Outgo	MgO Intake	MgO Outgo	K₂O Intake	K₂O Outgo	Na₂O Intake	Na₂O Outgo	Cl Intake	Cl Outgo
I. Low Ca and P	3641	6·8	16·4	15·4	30·0	21·4	18·4	76·3	22·6	—	6·7	—	9·3
	3648	6·8	11·7	15·4	21·7	21·4	1·3	76·3	16·1	18·9	4·8	51·4	6·6
II. Low P	3659	51·6	17·1	15·4	32·0	21·4	1·9	76·3	24·6	18·9	7·0	57·4	9·8
IIIA. Low Ca	3643	—	14·8	45·1	27·5	21·4	1·7	74·0	20·4	—	6·2	—	8·4
	3655	6·8	13·2	45·1	24·6	21·4	1·5	74·0	18·3	18·9	5·4	48·9	7·5
IIIB. Low Ca, Low Mg	3640	—	15·6	45·1	29·0	11·1	1·8	101·3	21·5	18·9	6·4	49·9	8·9
	3650	6·8											
IV. All min. low except Ca and P	3642	—	11·7	45·1	21·7	21·4	1·3	29·3	16·1	—	4·8	—	6·6
	3649	47·1	11·7	45·1	21·7	21·4	1·3	29·3	16·1	5·7	4·8	6·7	6·6
V. Low Na, Cl	3651	—	10·9	45·1	20·3	21·4	1·2	75·0	15·0	—	4·5	—	6·2
	3646	51·6	16·4	45·1	30·4	21·4	1·8	75·0	22·6	5·7	6·7	6·7	9·3
VI. Low Cl	3658	—	15·6	45·1	29·0	21·4	1·8	76·3	21·5	—	6·4	—	8·9
	3675	51·6	14·8	45·1	27·9	21·4	1·7	76·3	20·4	18·9	6·2	6·7	8·4
VII. Low Na	3653	51·6	17·2	45·1	32·0	21·4	1·9	76·3	23·6	5·7	7·0	57·4	9·8
VIII. Low K	3656	—	10·9	—	20·2	21·4	1·2	—	15·0	—	4·5	—	7·1
	3673	51·6		45·1		21·4		29·3		18·9		57·4	
IX. All min. sufficient + KI	3677	—	12·5	—	23·2	21·4	1·4	—	17·2	—	5·1	—	6·2
	3652	47·1	11·7	45·1	23·2	21·4	1·3	76·3	16·1	18·9	4·8	57·4	6·7
X. All min. sufficient	3645	47·1	12·5	45·1	23·6	21·4	1·7	76·3	17·2	18·9	5·1	57·4	7·1

TABLE IV.

Daily Mineral Intake in Feed and Outgo in Milk for Second lactation.

Group	D.O.B.	CaO Intake	CaO Outgo	P_2O_5 Intake	P_2O_5 Outgo	MgO Intake	MgO Outgo	K_2O Intake	K_2O Outgo	Na_2O Intake	Na_2O Outgo	Cl Intake	Cl Outgo
I. Low Ca and P	3641	14·8	—	17·4	—	18·1	—	81·0	—	27·5	—	—	—
	3648	14·8	—	17·4	—	18·1	—	81·0	—	27·5	—	63·3	—
II. Low P	3659	59·1	—	18·0	—	20·1	—	83·0	—	25·6	—	65·3	—
IIIA. Low Ca	3643	15·1	18·7	56·7	35·0	23·1	2·1	80·0	25·8	28·7	7·8	—	10·6
	3655	15·1	21·0	56·7	39·1	23·1	2·4	80·0	29·0	28·7	8·6	56·0	12·0
IIIB. Low Ca, Low Mg	3640	15·1	18·0	56·7	33·3	12·8	2·0	110·0	24·8	28·7	7·4	—	10·2
	3650	15·1	20·3	56·7	37·7	12·8	2·3	110·0	28·0	28·7	8·3	56·0	11·3
IV. All min. low except Ca and P	3642	55·4	17·2	56·7	32·0	23·1	1·9	38·0	23·6	15·5	7·0	13·8	9·8
	3649	55·4	18·0	56·7	33·3	23·1	2·0	38·0	24·8	15·5	7·3	—	10·2
V. Low Na, Cl	3651	59·9	—	56·7	—	23·1	—	80·0	—	15·5	—	13·8	—
	3646	59·9	—	56·7	—	23·1	—	80·0	—	15·5	—	—	—
VI. Low Cl	3658	59·9	—	56·7	—	23·1	—	80·0	—	28·7	—	13·8	—
	3675	59·9	—	56·7	—	23·1	—	80·0	—	28·7	—	—	—
VII. Low Na	3653	59·9	23·4	56·7	43·5	23·1	2·4	80·0	32·2	15·5	9·6	64·5	13·3
VIII. Low K	3656	59·9	—	56·7	—	23·1	—	38·0	—	28·7	—	64·5	—
	3673	59·9	—	56·7	—	23·1	—	38·0	—	28·7	—	—	—
IX. All min. sufficient + KI	3677	55·4	13·2	56·7	24·6	23·1	1·5	85·0	18·2	—	5·4	53·8	7·5
	3652	55·4	—	56·7	—	23·1	—	85·0	—	28·7	—	—	—
X. All min. sufficient	3645	55·4	18·0	56·7	33·3	23·1	2·0	85·0	24·8	28·7	7·3	64·5	10·2

From the above tables it is abundantly clear that 3641 was definitely suffering from a more acute phosphorus deficiency than 3648. The former animal actually secreted daily in the milk almost exactly twice as much phosphorus as was contained in the food. No wonder, therefore, that 3641 could not stand the conditions of the experiment at all.

With regard to calcium deficiency, the position was not much better, both animals receiving in the food only about half or less than half the calcium secreted in the milk. Both animals developed pica early in the experiment and were ferocious earth eaters whenever the opportunity was offered. Unfortunately the animals developed sore feet on the concrete floor where they were allowed to exercise, and had to be transferred to an adjoining sandy paddock for short periods on several occasions. The sand in this paddock contained ·2 per cent. CaO and ·0003 per cent. P_2O_5. Some of the phosphorus and calcium in the sand went to rectify the shortage of these two minerals, especially the Ca in the rations. Furthermore, the animals were given tapwater which ensured an additional intake of CaO per day of ·7 gm. The fact remains, however, that a calcium deficiency, although not recognisable clinically, most probably existed in this experiment as well as a definite aphosphorosis.

Table V gives the monthly milk yield of all the animals during both lactation periods in this experiment. Animal 3641 showed remarkable drops in the successive monthly milk yields. This decrease was not noticed in the smaller production of 3648 or in the control.

FIGURE 1

No. 3659.   Normal.   No. 3641.
Low P.   Animal.   Low Ca and Low P.

Reproduction of X-ray photographs of selected bones of 3641 and of the control are given below and throw light upon the experimental condition from another angle. For convenience, bones of animal 3659 discussed in Experiment II have been included (see Figures 1 and 2) and will be considered along with those of the control animal and of 3641.

FIGURE 2.
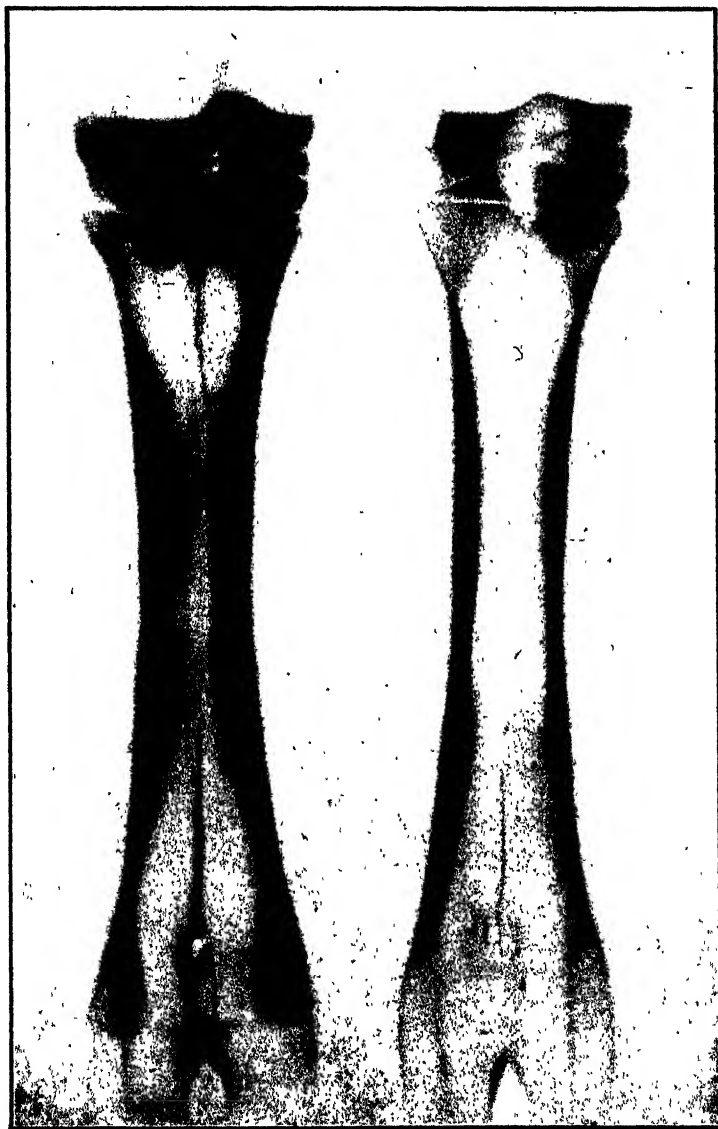
Normal.
Animal.

No. 3641.
Low Ca and Low P.

* The normal tarsal and metatarsal bones show a dense and well-defined cortex, the outline of which is very sharply marked off from the medullary cavity. The medullary cavity is smaller than is the case in the experimental animal owing to the greater development of the compact tissue. The epiphysial trabeculae are well marked. The junction of the third and fourth metatarsal bones is shown by a dark line at both extremities, which disappears toward the middle third of the bone. The greater density of the tarsal bones is shown by the darker shade in the photograph.

In the experimental animal these bones show a narrower and less dense cortex with a lack of definition in outline. The medullary cavity is larger and the spongy bone at the epiphysis not so dense. The tarsal bones also show a lighter shade in the photograph due to being less dense.

The Os Calcanei.

The contrast in density of the os calcanei in the control and experimental animals is very well illustrated in the photographs. The shadow in the bones of the control animal is much deeper. The cortex of the bones of the experimental animal is much more transparent and lacks a definite border line.

The os calcanei is an excellent bone in which to demonstrate the difference in bone density. The darker shadow shown by the more dense bone of the control animal is quite apparent from the photograph.

Experiment II.

Low Phosphorus but adequate in Other Respects.

History.

	No. 3659.	No. 3645 (control.)
No. of animal......................	No. 3659.	No. 3645 (control.)
Date experimental ration began beginning first gestation......................................	15.9.30	29.10.30
Date of calving................................	21.6.31	19.7.31
End of lactation...............................	21.9.31	19.10.31
End of rest period beginning second lactation......	14.11.31	14.12.31
Date of calving................................	23.8.32	25.9.32
Date of conclusion of experiment................	Died 4.10.32 (weakness and exposure).	25.12.32

The basal ration was in complete agreement with that of Experiment I, except that calcium carbonate was added to increase the daily intake of Ca to $47 \cdot 1$ gm.—The same as that of the control animal, No. 3645. The weight curves are given in Graph III. Graphic representations of the inorganic phosphorus content of the blood are given in Graph IV.

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* A description of the radiograms was kindly given by J. Quinlan, F.R.C.V.S., D.V.Sc., etc.

GRAPH IV.—Blood Analysis.

GRAPH III.—Weights of Animals in Pounds.
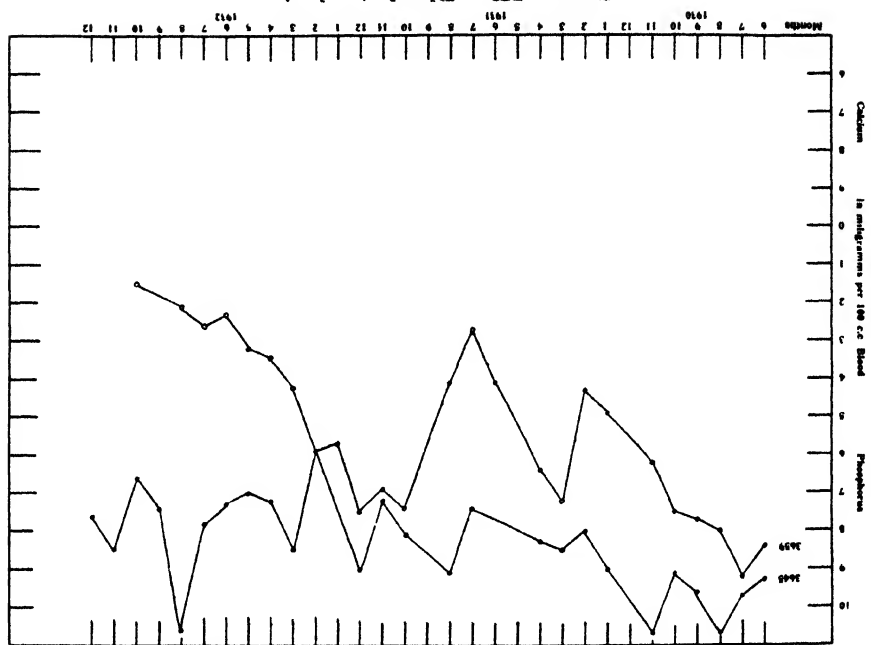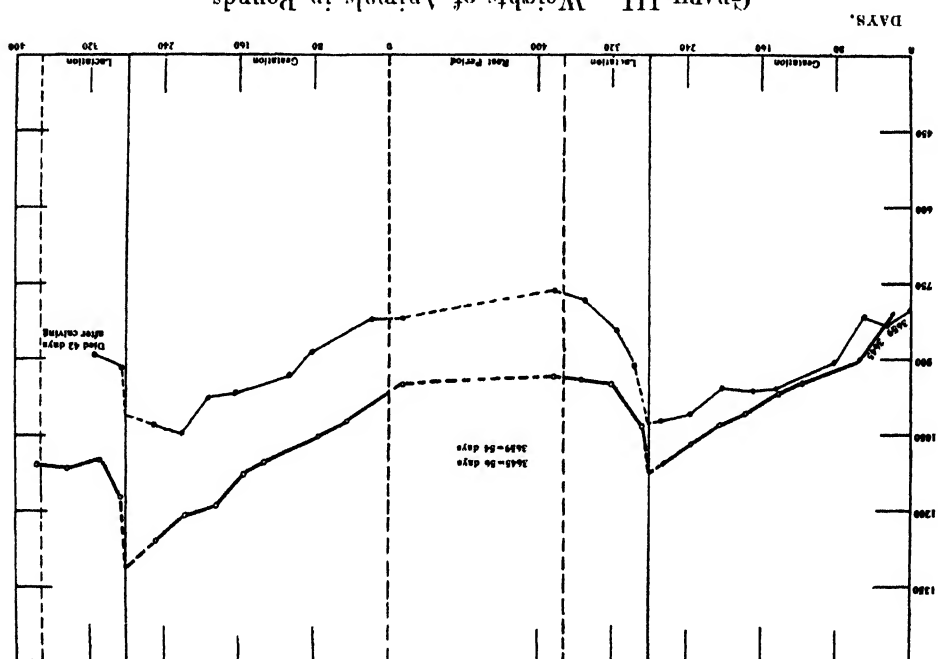
A study of the Graphs III and IV given above reveals that in many ways the course of this experiment follows that of the first experiment very closely. An appreciable difference in weight between 3659 and the control 3645 appeared only when the first lactation period began. The rest periods are about equally long and 3659 showed signs of improvement which continued until shortly before the second calving, when the animal began to drop in weight; her condition, already very poor, became worse as she was a heavy milker. Styfsiekte developed early in the experiment and became worse during lactation. Just over a month after calving the animal, then in a miserable condition, was unable to rise and was unfortunately left exposed to wind and rain during the night when she died. A post-mortem examination revealed a broken pelvis and several broken ribs. The bones were kept for pathological study at a later date.

The monthly blood analyses for inorganic phosphorus confirmed the observation made in regard to the weight curves. During the rest period the animal showed a normal value for blood phosphorus which rapidly dropped in February, 1932, when fanko was substituted for maize as in Experiment I. Low inorganic phosphorus in the blood continued until the end of the experiment.

The daily intake of minerals in the ration and output in the milk is represented in Tables III and IV.

It is apparent from Table III that No. 3659 was actually secreting more than twice the amount of phosphorus in her milk than she was getting in her food. No wonder, therefore, that her milk production during the third month was markedly less than that of the first month ($\pm$ 200 lb. less). The phosphorus deficiency in addition to the protein shortage must have made the position almost unbearable during lactation or perhaps protein shortage helped to limit milk production and in that way saved the animal from further depleting her system to supply the necessary phosphorus. At all events phosphorus deficiency during lactation was an established fact. From Graph IV it is evident that phosphorus deficiency existed not only during lactation but also towards the end of the first gestation period, and from February, 1932, when fanko was increased at the expense of maize, until the animal's death.

Calcium was abundantly present throughout, and blood calcium did not appear to be different from that of the control or from that of the animals in Experiment I.

Complete data with regard to the mineral content of the blood of the bovines for the full period of the investigations reported on in this paper will be found in the publication by Groenewald (1934). These analyses are omitted from this article for reasons of economy and to obviate repetition.

A comparison of Experiments I and II leaves doubt as to what the effect of low calcium was in the former experiment. The history of animal No. 3641, a high producing cow, in Experiment I on low Ca and P, follows that of No. 3659, also a high producing cow on low phosphorus only, in Experiment II, pretty closely, both having stood the strain of the first lactation period with loss in weight,

development of acute styfsiekte and general setback. The strain of the second gestation period was too severe and both succumbed about a month after the birth of the second calf. A comparison of these two experiments with No. III will be made after discussion of the latter. For the present it can only be said that the effects of a phosphorus deficiency on animal No. 3659 was as fatal as that of a phosphorus deficiency plus low calcium on No. 3641—two animals directly comparable as regards milk production.

EXPERIMENT III.

Two pairs of heifers were included in this experiment, (A) one pair was placed on low calcium ,and high magnesium), while (B) the other pair received both low calcium and low magnesium. The higher magnesium in A incidentally improved the Erdalkali-alkalizität in Marek's sense, while the magnesium was kept low in the other case, although hardly sufficiently so to be of any significance in an attempt to approach Becka's idea (1929) of the good effect of magnesium upon calcium metabolism. Reference to Table II shows the intake of CaO to be 6·8 gm. daily and MgO 21·4 and 11·1 gm. respectively.
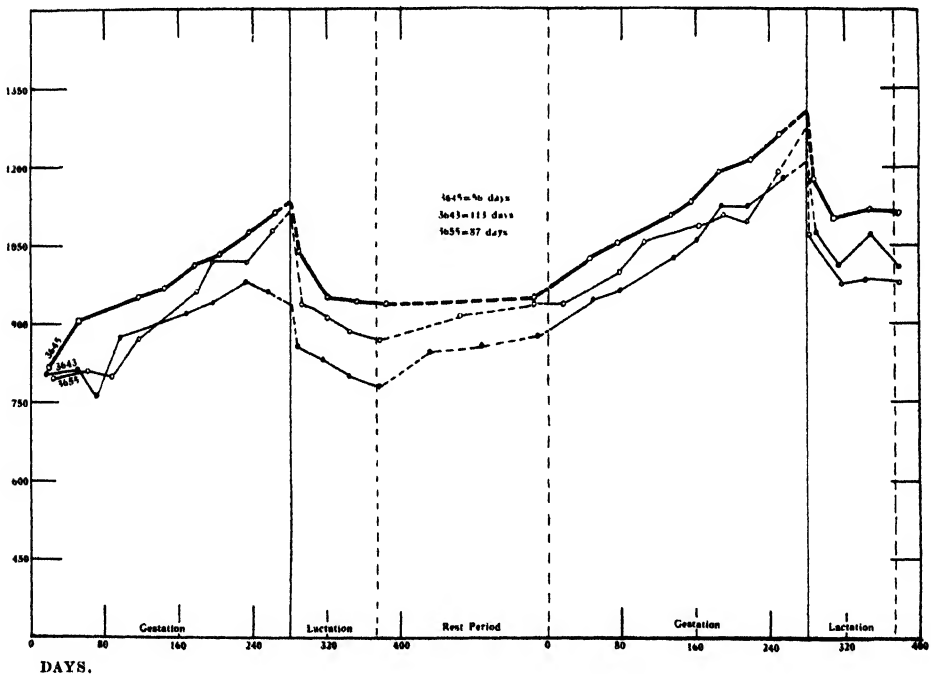
GRAPH V.—Weights of Animals in Pounds.

History.

No. of animal............	No. 3643.	No. 3655.	No. 3649.	No. 3650.	No. 3645. (control.)
Date experimental ration began, i.e. beginning first gestation..............	2.9.30	1.9.30	9.9.30	28.8.30	29.10.30
Date of calving.........	27.5.31	24.5.31	2.6.31	1.6.31	19.7.31
End of lactation........	27.8.31	24.8.31	2.9.31	1.9.31	19.10.31
End of rest period, i.e. beginning second lactation	18.12.31	19.11.31	13.11.31	not pregnant	14.12.31
Date of calving.........	22.9.32	25.8.32	21.8.32		25.9.32
Date of conclusion of experiment.................	22.12.32	25.11.32	21.11.32	15.12.32	25.12.32

A graphic representation of the weights of (A) the animals on low calcium only, viz., Nos. 3643 and 3655, and (B) the animals Nos. 3640 and 3650 on low calcium and low magnesium, are given in Graphs V and VI respectively.

3645 ~ 56 days
3640 ~ 72 days
3650 ~ 72 days

DAYS.
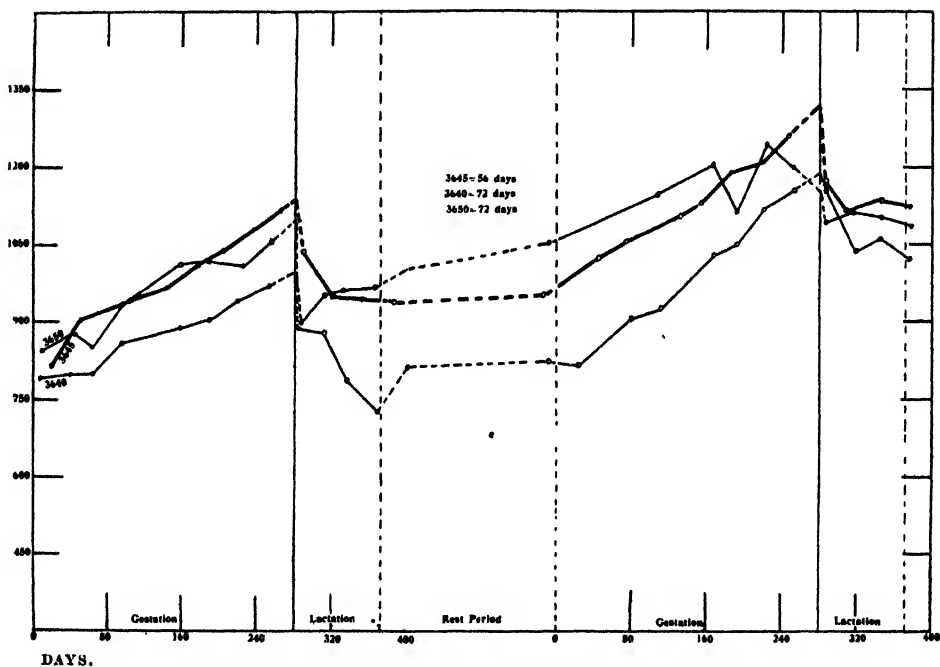
GRAPH VI.—Weights of Animals in Pounds.

Before comparing Graphs V and VI it must be noted that animal No. 3640 calved after the first gestation period, but dried off within a week. It is not surprising, therefore, that she remained in excellent condition until shortly before the end of the second gestation period when she began to lose weight. Still, after 3 months lactation, this animal finished almost on a par with the control, No. 3645. No. 3650 felt the drain of lactation severely in 1931, but for the rest nothing very significant is apparent from the charts, except perhaps that the second lactation period gradually increased the difference

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between No. 3650 and the control. The differences between No. 3643 and No. 3655 (Graph IV) are perhaps even less significant. It is true that these animals were lighter than the control, but such was the case practically from the beginning of the experiment, and only during the two lactation periods was the difference between the control on the one hand and the experimental animals on the other made significant. It seems, therefore, that the low calcium that existed in the rations of the animals in this experiment might have made itself felt in a longer lactation period but that for the rest of the experimental period it did not show significant effects upon the animals.

It is impossible to state definitely the effect of low or high magnesium upon the animals. Nos. 3643 and 3655 seem to have done slightly better on the whole, but generalizing would be dangerous without further proof. The effects of a calcium deficiency during lactation—the only time that such a deficiency was certain—undoubtedly masked the effect of magnesium. At all events whether high or low magnesium was present, the curves diverge during lactation, i.e. the period of acute Ca deficiency and show a tendency to converge gradually for the rest of the period.

It would be interesting to glance at the milk production of these four animals given in Table V and also at the Tables III and IV giving the intake of minerals in the food and the outgo in the milk.

Undoubtedly greater milk production by the four experimental animals made greater demands upon their systems than in the case of the control animal. Proof of this lies in the weight chart of No. 3655 during the first lactation period. She approached the control more closely than her mate but produced about the same quantity of milk as the control and well over 200 lb., i.e. more than 2 lb. a day less than her mate. During the second lactation period when a protein supplement was given, and incidentally the calcium intake more than doubled, although it remained still definitely less than the output in the milk, the differences of the effect of lactation upon the weight curve in the case of No. 3650 and No. 3640 was even less marked. No. 3640 produced about the same quantity of milk as the control and No. 3650 just over 2 lb. a day more. Both Nos. 3655 and 3643 had difficulty at calving the second time, went off their feed and were reported sick for several days. This additional setback must be borne in mind when the weight curves are studied.

That longer lactation periods would have made themselves felt and would have had marked effects upon the weights of the experimental animals, but that, for the rest of the experimental period, the low calcium in the ration had doubtful effects upon the animals, seems to be suggested by the weight charts of the animals and by a consideration of the intake and outgo of minerals during lactation. This is apparently a justifiable conclusion. Furthermore it is well to remember that soil eating could not be entirely eliminated from this experiment, and that the intake of calcium was, therefore, slightly more than that stated in Table II.

The results of the blood analyses for Ca and Mg of Nos. 3643, 3655 and 3645 and of Nos. 3640, 3650 and 3645 do not reveal anything significant.

Both calcium and magnesium remained remarkably constant throughout the experimental period. Apparently the calcium in the food was not sufficiently low to produce an effect upon blood calcium, while it is doubtful whether a daily intake of 11·1 gm. MgO could be regarded as insufficient for the requirements of cattle. In any case, the magnesium level of the blood remained remarkably constant throughout.

A comparison of the experiments so far considered, viz. low calcium with high and low magnesium respectively; low calcium and low phosphorus; and low phosphorus, is necessary at the present stage, and brings to light several salient factors.

It is difficult to see what rôle low calcium played in these experiments. The effects of low calcium and low phosphorus were not more detrimental to the animal than those of low phosphorus alone. The demand for phosphorus during milk production was higher than that for calcium and apparently the animals in the low calcium groups were able to meet that demand without serious loss of condition, while that in the phosphorus low group died indirectly of aphosphorosis. It seems that 11·1 gm. MgO were enough to meet the demands of the animals for that mineral. It is realized that the figure giving the intake of calcium in Tables II, III and IV is probably slightly lower than the actual intake; still it is thought that a serious calcium deficiency would be difficult to bring about except during lactation.

Practical aspects of this work upon low calcium and low phosphorus will be considered further on in the text.

EXPERIMENT IV.

Ration low in all minerals except P and Ca.

History.

No. of animal	No. 3642.	No. 3649.	No. 3645 (control).
Date of experimental ration began, i.e. beginning first gestation	15.9.30	21.8.30	29.10.30
Date of calving	10.6.31	12.5.31	19.7.31
End of lactation	10.9.31	12.8.31	19.10.31
End of rest period, i.e. beginning second lactation	6.11.31	20.11.31	14.12.31
Date of calving	16.8.32	25.8.32	25.9.32
Date of conclusion of experiment	16.11.32	25.11.32	25.12.32

Reference to Table II shows that the intake of minerals for the greater part of the experiment was as follows:—CaO 47·1 gm., MgO 11·1 gm., K_2O 29·3 gm., Na_2O 5·7 gm., P_2O_5 45·1 gm., Cl 6·7 gm., SO_3 12·9 gm. The ration is potentially alkaline with a decidedly positive earth-alkali-alkalinity.

The weight charts of the animals in this experiment, viz. Nos. 3642 and 3649 are given in Graph VII together with that of No. 3645.

Both these experimental animals were lighter at the beginning of the experiment than the control and both showed very remarkable drops in weight during the first lactation period, although they had
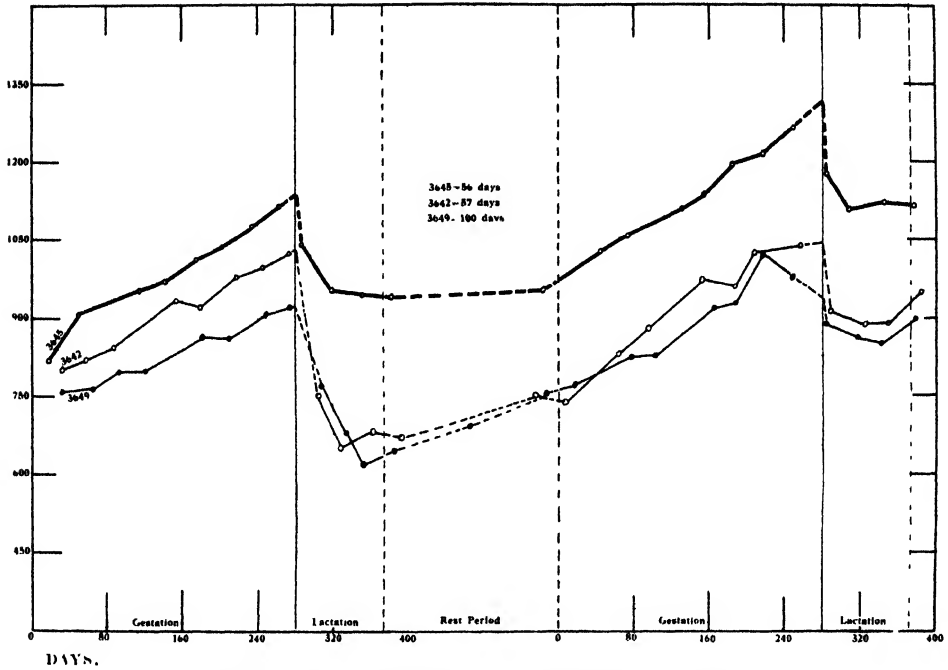
GRAPH VII.—Weights of Animals in Pounds.

kept condition quite well up to the time of calving. After that they remained in very poor condition throughout the experiment, were poor at calving and could ill afford a further drop in weight. They showed poor appetites for the first time during the first lactation period and repeated this behaviour during the second lactation period. On an average each consumed daily a lb. of the maize-fanko mixture less during both lactation periods than she did otherwise or than the control animal did. Food was refused eratically, all or practically all the food being refused approximately once a week during lactation. Apart from their poor condition the animals appeared listless most of the time and were reported stiff on several occasions.

The animals showed a decided improvement during the rest period, but it seems that gestation heavily taxed their powers of endurance, and that a change for the worse set in before the end of gestation. A second lactation period was just about as much as the animals could stand, although it must be noted that they gradually increased in weight towards the end of lactation, of which more anon.

Both animals produced approximately as much milk as the control as shown in Table V.

It is noteworthy that the milk flow rapidly decreased in both cases during the first lactation period, the animals giving only about a third of the first months' milk during the last month. As a matter of fact, the reason why it was decided to discontinue milking after

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3 months in all the groups, was because Nos. 3642 and 3649 had practically ceased to produce milk at the end of that period and were very rapidly drying off. In both cases less than 2 lb. milk per day were produced during the last week against 24 lb. in the beginning. This is also the probable reason for the slight increase in weight during the latter part of the first lactation period. During the second lactation period milk production continued steadily for the full period and a glance at Tables III and IV giving the intake of minerals and their secretion in the milk, supplies the reason for the difference in this respect between first and second lactation.

Table III refers to the first lactation period when the animals were on a protein deficiency as well as a deficiency of certain minerals. Magnesium again, may probably be ruled out as the intake was 21·4 gm. and only 1·3 gm. secreted in the milk. It is difficult to state anything definite about potassium at this stage. The daily intakes of sodium and chlorine during the first lactation period were 5·7 and 6·7 gm. respectively, while the quantities secreted in the milk were respectively 4·8 and 6·6 gm. Both elements were dangerously low in the food and could not have met the demands of the animals for these minerals for both milk production and other requirements. Referring to Table IV it is seen that the position with regard to the intake of minerals had improved greatly. Magnesium and potassium with no or in any case only doubtful deficiency during the first lactation period is more or less unchanged but more than twice the sodium contained in the milk was supplied in the food while the change was not quite so favourable as regards chlorine.

However, the effect of an all round better supply of minerals during the second lactation period is reflected in the weight curves, the animals showing only a small initial drop due to milk production and actually increasing in weight during lactation. One is forced to conclude that with protein sufficiency the intake of minerals in the case of Nos. 3642 and 2649 was enough to justify a better level of nutrition than that which existed when the animals calved. Hence an increase in weight soon set in. The milk production was on a par with that of the control. Bearing in mind the marked and rapid decrease in milk production during the first lactation period and the fact that protein deficiency did not have as marked an effect on milk production in any of the other groups, one is forced to the conclusion that the low intake of minerals other than calcium and phosphorus was rapidly felt and effected a decrease in milk which ensured a more favourable balance of intake over outgo during the last month of lactation than during the first.

Two points stand out clearly. A deficiency in minerals other than phosphorus or calcium is not easily brought about as shown by the second lactation period when the intake was not very greatly in excess of the mineral content of the milk, but still milk production continued normally and the animals were gaining weight, i.e. the mineral intake supplied the demand for milk production and other physiological requirements. When such a deficiency is effected, however, as for instance during first lactation, milk flow is rapidly decreased and one is led to presume that the body reserves of these minerals are not extensive and are rapidly exhausted.

There can be no doubt, however, that animals Nos. 3642 and 3649 were adversely affected by the conditions of the experiment. Both compared unfavourably throughout the experiment with the control. Not only were they lighter in weight, but to the eye they were poorer in condition, listless, had a rough coat, and appeared abnormal. That either sodium or chlorine or both contributed their share to the detrimental effects of the rations, seems safe to assume, but it is doubtful whether these were the only operating factors. When the next experiment is considered this point will be referred to again.

There are no outstanding points in the results of the blood analyses. As could be anticipated blood phosphorus indicates suffi-ciency throughout the experiment. Apparently the levels of the other minerals in the blood are not appreciably affected by the pro-portions of the minerals present in the rations of the animals under discussion. The high potassium value in the blood of No. 3642 was noticed right from the beginning of the experiment and it remained so throughout.

EXPERIMENT. V.

Ration low in Sodium and Chlorine but adequate in Other Respects.

History.

	No. 3651.	No. 3646.	No. 3645 (control).
No. of animal			
Date experimental ration began, i.e. beginning first lactation..........	9.9.30	21.8.30	29.10.30
Date of calving...................	4.6.31	21.5.31	19.7.31
End of lactation..................	4.9.31	21.8.31	19.10.31
End of rest period, i.e. beginning second lactation........................	not pregnant.	died 18.12.31	14.12.31
Date of calving...................	—	(impact).	25.9.32
Date of conclusion of experiment......	30.11.32	—	25.12.32

Until May, 1932, the daily intake of Na and chlorine was 5·7 and 6·7 gm. respectively, whereas the other minerals were present in adequate quantities as in case of the control.

Graph VIII gives charts of the weights of animals Nos. 3651 and 3646 on a diet low in sodium and chlorine compared with the weight curve of the control No. 3645.

A glance at Graph VIII reveals the fact that comparisons can be made only until the end of the rest period. One of the experi-mental animals then died while the other did not conceive and therefore had no second calf. The weight curves of Nos. 3646 and 3651 suggest that detrimental effects of the experimental ration began to show about a month and a half before calving. The one animal actually dropped in weight while the other remained constant. Lac-tation accelerated the weight loss and it would be well therefore to glance at the milk production given in Table V at this stage.

Both the experimental animals started salivating excessively about 6 months after the beginning of the experiment, i.e. approxi-mately three months before calving (9.2.31). This condition was
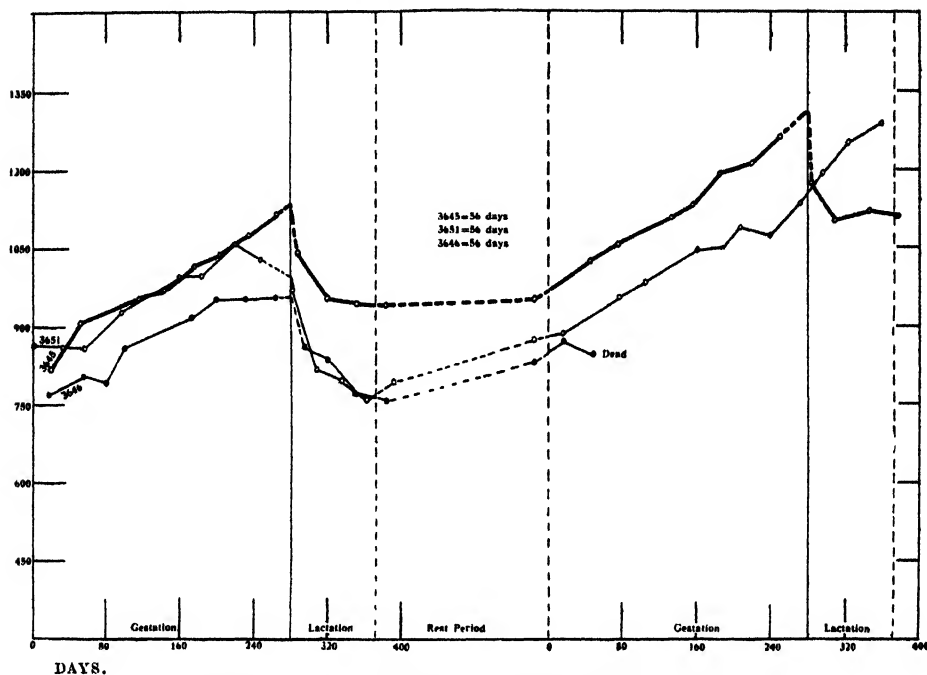
GRAPH VIII.—Weights of Animals in Pounds.

most noticeable during the hot hours of the day when saliva would be running freely for hours at a stretch, while breathing took the form of short, quick movements. In the case of animal No. 3646 this condition lasted at intervals of several days until the animal was removed to hospital on 30.11.31. At that stage the animal ceased eating and defecating, although up to that time she had not refused any of her food on a single occasion. The animal became worse, was given a purgative, without effect, and died 11 days afterwards of impaction of the omasum. This animal was a good milker and produced several hundred pounds milk more than either her companion or the control.

No. 3651 also began salivating profusely approximately 6 months after the beginning of the experiment. This condition was never so acute as in the case of No. 3646, and disappeared completely at the end of lactation. This animal did not conceive a second time and was discharged from the experiment towards the end of 1932 in prime condition.

There seems little doubt from the figures given in Table III, however, that these two animals suffered from a sodium chloride deficiency during lactation, if it is remembered that No. 3646 was actually secreting more sodium and chlorine in her milk than she was getting in her feed, while No. 3651 was secreting only just a little less. It is surprising that the effects of a sodium chloride deficiency were not more noticeable for, apart from profuse salivation and abnormal respiration, the one animal, at all events, behaved

normally, while it is uncertain in how far the death of the other animal can be associated with the deficient diet. At all events this experiment is indicative of interesting results when working with rations very low in sodium and chlorine, while the possibility of such deficiency during periods of poor feeding, as for instance during droughts, should be kept in mind. This point will again be considered under a general discussion of the significance of mineral deficiencies. A comparison of Graph IX giving the weights of animals on a ration taken to be low in all minerals except Ca and P, with Graph X, leaves doubt about the ultimate effect of low Na and Cl in the ration. Na and Cl deficiency undoubtedly existed during lactation, but not with the same detrimental effect as in the last experiment, although the milk production of No. 3651 decreased rapidly, it is true. However, the point remains that apparently the bad effects of the experimental conditions in Experiment IV were not due to Na and Cl deficiency only or alternately other conditions in Experiment V, e.g. abundant K, partly masked the effects of low Na and Cl. It seems necessary to elucidate this point by conducting an experiment with cattle on low Na and Cl with low K and high K, respectively.

The blood analysis for sodium was without significance, the experimental animal showing figures that were no higher than those of the control. In the case of chlorine, especially during the period of acute deficiency, i.e. during the latter portion of the first gestation period and the whole of the lactation period a decided drop was shown as Graph IX suggests. It seems that low chlorine in the ration might be reflected as low chlorine in the blood. Such a condition seems natural if it is remembered that a low chlorine intake results in decreased elimination of chlorine via the kidneys—a condition probably associated with a lower chlorine level in the blood.

EXPERIMENT VI.

Ration low in Chlorine but adequate in Other Respects.

History.

No. of animal	No. 3658.	No. 3675.	No. 3645 (control).
Date experimental ration began, i.e. beginning of first gestation.	3.9.30	12.8.30	29.10.30
Date of calving.	7.6.31	12.5.31	19.7.31
End of lactation	7.9.31	12.8.31	19.10.31
End of rest period, i.e. beginning of second gestation	—	9.1.32	14.12.31
Date of calving.	—	11.10.32	25.9.32
Date of conclusion of experiment	died 19.8.32 (T.B.)	died 29.10.32 (peritonitis.)	31.12.32

The daily chlorine intake was 6·7 gm. against 57·4 gm. in case of the control.

The weight curves of the experimental animals Nos. 3658 and 3675 together with that of the control are given in Graph IX.

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3645=56 days
3658=86 days
3675=150 days

Aborted.
Lactated 50 days

Gestation   Lactation   Rest Period   Gestation   Lactation

DAYS.
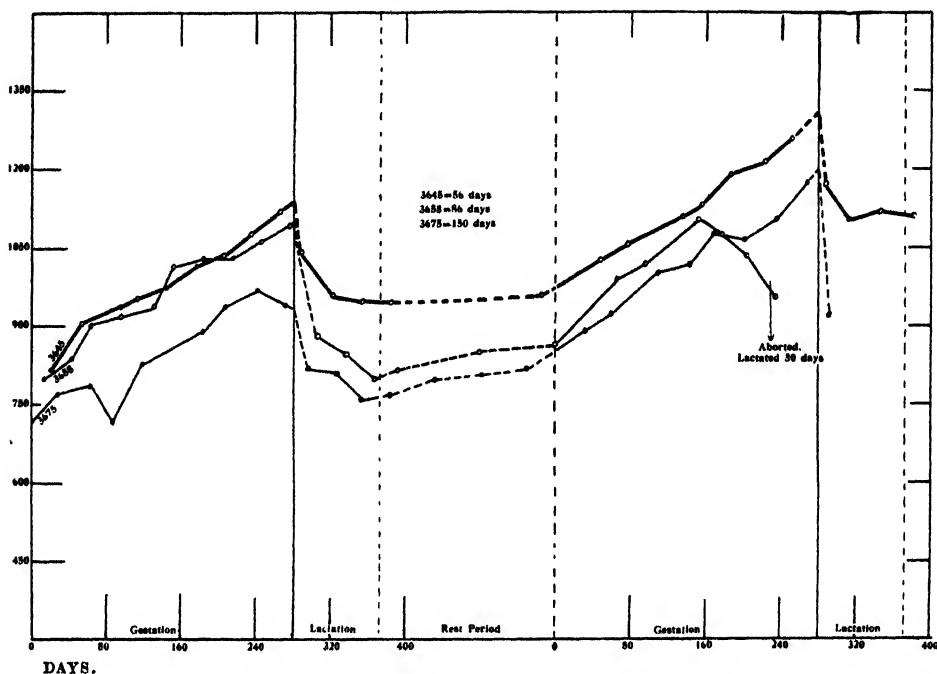
GRAPH IX.—Weight of Animals in Pounds.

The milk production of the two experimental animals and of the control is given in Table V, while the intake of minerals and the outgo in the milk are given in Tables III and IV.

Both experimental animals actually secreted more chlorine in their milk during the first lactation period than that contained in the food—a condition which must have affected the animals adversely. It is also noticed, however, that the experimental animals were heavier milkers than the control, each having produced at least 3 lb. of milk more daily. These two groups, viz. chlorine deficiency and heavy milk production, must be borne in mind when viewing the greater decrease in weight of Nos. 3658 and 3675 during lactation than of No. 3645. Unfortunately both animals lived only for a short while after calving the second time; No. 3675 died of peritonitis after puncture of the uterus, while No. 3658 had to be destroyed on account of tuberculosis infection. Still the fact remains that, apart from lactation when, with greater secretion of chlorine in the milk than the intake of this constituent in the food, it is doubted how far the low chlorine of the ration had any bearing upon the course of the experiment. The rate of increase of weight of Nos. 3675 and 3658 agrees closely with that of the control, although the latter was and remained a heavier animal than No. 3675. No clinical symptoms were noticed in either animal during the experiment, food consumption was normal, and the animals behaved normally in all respects.

The results of the blood analyses for chlorine seem to confirm the findings in regard to the chlorine level of the blood made in the

last experiment, viz., that low chlorine in the ration is associated with low chlorine in the blood.

Summarizing the details of this experiment, it cannot be said that low chlorine, such as it was, affected the animals very adversely. Growth was satisfactory except during lactation. The average daily milk production showed a greater loss of Cl in the milk than that contained in the food, but both animals produced only about 12 lb. milk daily during the latter part of lactation, or in other words, less chlorine was secreted than was actually contained in the food. Apparently even a very low Cl intake did not have a marked effect upon the animals in this experiment taken generally.

Experiment VII.

Ration low in Na but adequate in Other Respects.

History.

No. of animal......................	No. 3672.	No. 3653.	No. 3645 (control).
Date experimental ration began, i.e. beginning of first gestation.........	20.8.30	26.8.30	29.10.30
Date of calving.....................	10.5.31	24.5.31	19.7.31
End of lactation....................	10.8.31	24.8.31	19.10.31
End of rest period, i.e. beginning of second gestation..................	13.11.31	8.11.31	14.12.31
Date of calving.....................	—	18.8.32	25.9.32
Date of conclusion of experiment.....	discharged 16.5.32 (J.B.)	30.11.32	31.12.32
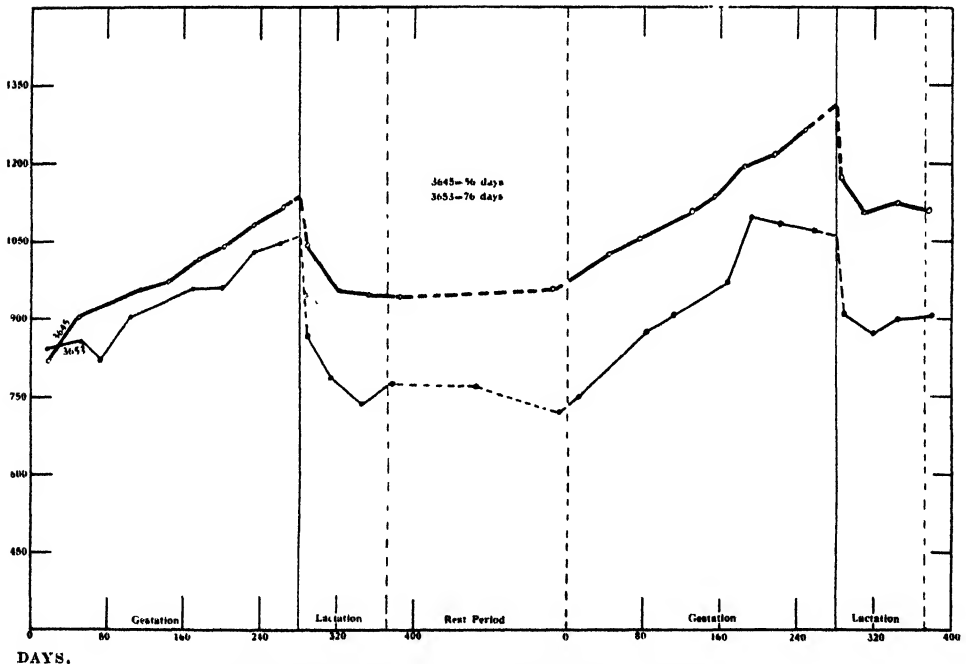
Graph X.—Weight of Animals in Pounds.

The Na intake of the two bovines No. 3672 and No. 3652 was 5·7 gm. as compared with 18·9 gm. in case of the control.

The weights of animal No. 3653 is given in Graph X.

As animal No. 3672 was severely infected with tuberculosis, and showed many lesions on the post-mortem table, she will not be considered in the discussion of this experiment.

The milk production is given in Table V, while details of the difference between the intake of sodium and outgo in the milk are given in Tables III and IV.

It is evident that No. 3653 was a much heavier milker than the control, having produced during the first lactation period 2,127 lb. milk as against 1,446 lb. by the control. Furthermore, the outgo of sodium in the milk during lactation was definitely greater than the intake in the food. However, during both the first gestation and portion of the second gestation periods the animal did well, increasing in weight. Towards the end of the second gestation period, however, No. 3653 began to lose weight, which continued until after calving.

Considering the greater milk production of No. 3653 when compared with No. 3645, it is difficult to gauge the effect of low sodium in the ration. During lactation low sodium undoubtedly had some effect upon the animal as the excess of outgo over intake had to be provided for from the body reserves. The animal went off her feed on several occasions, appeared listless and lay down most of the time about a month before giving birth to her first calf. During the second gestation period she ate well and appeared normal throughout. It does seem probable, however, that towards the end of gestation the sodium deficiency began to be felt, if weight decrease is a criterion at all.

The blood analyses for sodium shows no outstanding difference between the sodium level in the blood of the control compared with that of the experimental animal.

The practical significance of low sodium in the diet of growing bovines is doubtful. Whether it is possible to obtain a ration sufficiently low in sodium to make itself felt, is also doubtful, except during lactation. It is remarkable that a ration containing only 5·7 gm. sodium daily could produce a normal increase in weight until lactation, and again afterwards, when lactation ceased, without causing more devastating effects than appeared in bovine No. 3653 in the experiment under review. However, it is to be admitted that the course of the experiment was not quite normal. Milk production was reduced during the first lactation period from 33 lb. daily during the first month to about 15 lb., which, incidentally, meant that during the latter part of lactation slightly more sodium was contained in the food than in the milk. On the other hand, the converse was the case for the first two months of lactation, and the animal lost heavily in weight. The second gestation period was not uneventful and the drop in weight towards the end shows that the strain of gestation began to be felt. During the second lactation period, however, the animal showed almost complete recuperation, at all events a great improvement, and a glance at Table IV reveals that the sodium intake was about 1½ times the quantity secreted in the

milk. Milk production was very high—the highest of all the animals —but in spite of this the animal actually gained in weight. It appears that, although 5·7 gm. Na₂O was insufficient for the requirements of a 2-gallon cow during lactation and during the latter part of gestation, 15·5 gm. was definitely enough for a 3-gallon cow.

<center>EXPERIMENT VIII.</center>

<center>*Ration low in K, but adequate in Other Respects.*</center>

<center>*History.*</center>

No. of animal......................	No. 3656.	No. 3673.	No. 3645 (control).
Date experimental ration began, i.e. beginning of first gestation.........	19.8.30	29.8.30	29.10.30
Date of calving...................	8.5.31	5.6.31	19.7.31
End of lactation..................	Died 1.6.31 (metritis)	5.9.31	19.10.31
End of rest period, i.e. beginning of second gestation..................	—	29.4.32	14.12.31
Date of calving.............	–	6.2.33	25.9.32
Date of conclusion of experiment.. ..	—	6.5.33	25.9.32

From Table II it is evident that the daily potassium intake was approximately 30 gm. while the rest of the minerals contained in the ration was the same as those of the control animal No. 3645.
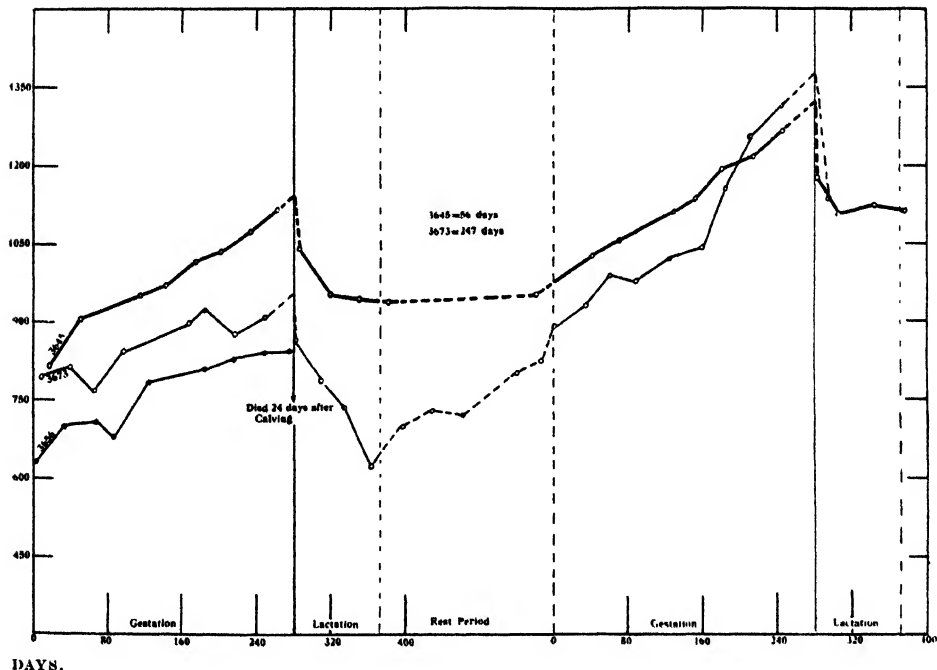
<center>GRAPH XI.—Weight of Animals in Pounds.</center>

<center>595</center>

The weight curves of No. 3656 and 3673 together with that of the control are given in Graph XI.

One of the experimental animals, viz. No. 3656, died of metritis 21 days after giving birth to a calf, while No. 3673 lasted the full period of the experiment, but with the longest rest period, viz., 247, days. This animal showed an appreciable drop in weight during the first lactation period, but improved in condition immediately afterwards until the second lactation began. Taking the weight curve as a whole, the only outstanding point is the great loss of weight during the first lactation period. After calving this animal suffered from mastitis, was removed to and treated in hospital for a considerable time. Hence it is difficult to judge the cause of the drop in weight correctly with three complicated factors, viz. low potassium, low protein and mastitis. During the second lactation, when the first two factors were absent, the response of the animal was quite normal compared with the control. At all events it appears that there was no deficiency present.

A glance at Tables III and IV, giving the intake in the food and the outgo in the milk of potassium during both lactation periods, and at Table V, giving milk production, confirms the tentative suggestion made by the weight curve, viz. that low potassium was probably not a serious factor to contend with in this experiment. Only about half of the potassium content of the food during both lactations was secreted in the milk. While a definite conclusion as regards the sufficiency or otherwise of potassium in the ration of the experiment under discussion cannot be drawn, there is no presentable proof for believing that potassium was deficient.

On the whole the potassium level of the blood of No. 3673 is slightly higher than those of the control. Whether these high values are significant, it is difficult to say, for the normal variation of K in blood is considerable. This point about the relation between the potassium in the food and that in the blood must be left in abeyance until more information is forthcoming.

EXPERIMENT IX.

Sufficiency of all Minerals plus a Supplement of Potassium Iodide.

History.

No. of animal	No. 3677.	No. 3652.	No. 3645 (control).
Date experimental ration began, i.e. beginning of first gestation	3.9.30	2.9.30	29.10.30
Date of calving	2.6.31	2.6.31	19.7.31
End of lactation	2.9.31	2.9.31	19.10.31
End of rest period, i.e. beginning of second gestation	12.12.31	11.12.31	14.12.31
Date of calving	19.9.32	10.9.32	25.9.32
Date of conclusion of experiment	19.12.32	died 18.9.32 (peritonitis and metritis)	25.12.32

The two animals Nos. 3652 and 3677 in this experiment were on the same ration as the control, but received in addition a supplement of 0·1 gm. KI daily. Both animals remained normal, and ate well. One was discharged towards the end of the experiment as a suspicious case of tuberculosis, while the other gave birth to twins, retained her afterbirth and died of metritis 28 days afterwards.

The weight curves of the animals in this experiment are given below in Graph XII together with that of the control No. 3645.

DAYS.
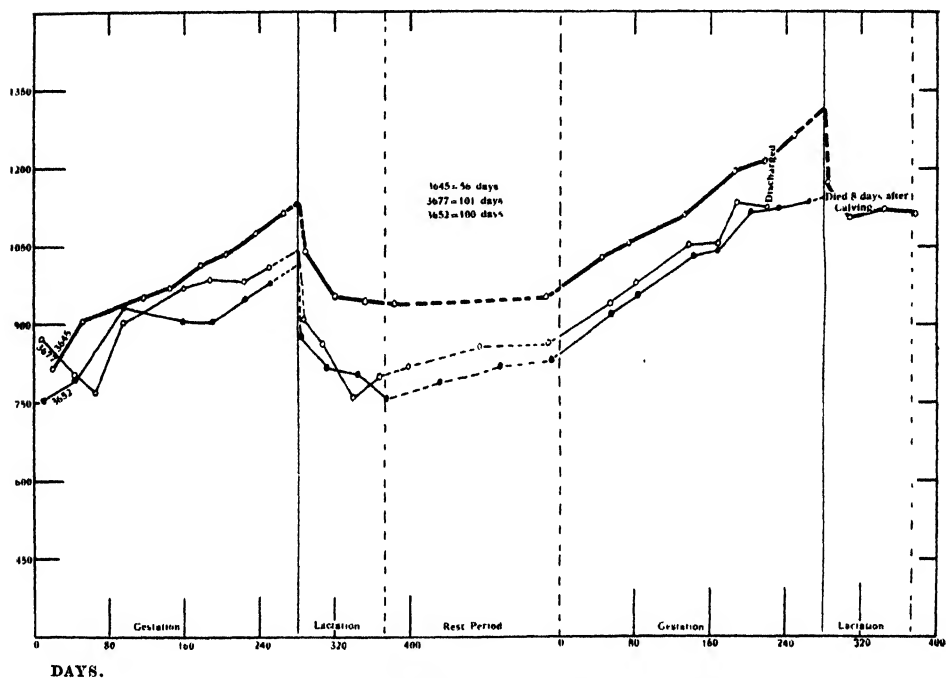
GRAPH XII.--Weight of Animals in Pounds.

Neither from daily observations of the animals nor from the weight curves were any beneficial effects of potassium iodide administration noticeable. The experimental animals were not as heavy as the control, but the rate of increase or decrease was never markedly different. The milk production is given in Table V.

The milk production of the experimental animals was approximately the same as that of the control. Both lost more weight during lactation than the control, but at the beginning of the second gestation period the difference in weight of the experimental animals compared with that of the control was about the same as at the beginning of lactation, and remained practically so until the end of the experiment.

No marked beneficial effects of the iodine in the ration were noticed, nor can it be said that the iodine showed harmful effects.

The milk of both experimental cows contained, as was anticipated and has often been shown (Crichton, 1930), considerably more iodine than that of the control. The following figures give an indication of the results obtained.

Iodine in daily milk of No. 3677 varied from 39 to 99 γ per 100 c.c., while that in the milk of No. 3645 receiving no KI in its feed showed an average value of $7 \cdot 2 \gamma$ $(5 \cdot 6-9 \cdot 2)$.

No practical benefit of iodine feeding has been observed in this experiment. Perhaps a larger experiment carried out under field conditions to make differences in milk, production, reproduction, keeping conditions, etc., significant, would be a fairer test of the beneficial effect or otherwise of iodine feeding than the one reported on here.

V. GENERAL DISCUSSION OF EXPERIMENTS AND COMPARISON OF THE RESULTS OBTAINED.

It is undoubtedly difficult to assign correct values to the rôle of the respective minerals in nutrition, in the light of the results of the foregoing experiments. A number of salient points have been brought out by this investigation, however, and it is proposed to deal in this discussion with such factors and to compare the results of the experiments in a general way.

On the whole the results of individual experiments, with few exceptions, have been neither decidedly positive nor negative. This fact is most remarkable if Table II, on page 570, be looked at carefully. The table reveals the fact often repeated in this report that, with the single exception of magnesium, the intake of the minerals intended to be deficient in any particular experiment, was remarkably low. As a matter of fact, the intake was so low that, with the current conception that only a small proportion of minerals present in an ordinary ration (Crichton states 15–20 per cent.) is absorbed, it is remarkable that the animals could produce milk at all. In most cases they required daily approximately as much, and in several cases more, of the particular mineral (Ca, P, Na, K and Cl) than the ration contained of that particular constituent. Yet milk production continued under those conditions for 90 days, with, it is true, in some cases bad effects upon the animal, but no fatal effects except in the phosphorus deficient group or complete cessation of milk flow except perhaps in Nos. 3642 and 3649, when an attempt was made to make all minerals, except Ca and P, as low as possible. Drastic effects of the mineral intakes during lactation upon the animals would be anticipated from the fact that in all cases except magnesium, the total daily intake of a particular mineral was hardly different from that secreted in the milk during the same period apart from other physiological requirements of the animals.

At this stage it is necessary to study Tables III, IV and V carefully.

TABLE V.

Monthly Milk Yield in Pounds.

D.O.B. No.	Group	First Lactation, 1931.				Second Lactation, 1932.			
		Month.			Average per day in lb.	Month.			Average per day in lb.
		1st.	2nd.	3rd.		1st.	2nd.	3rd.	
3641	I. Low Ca and P.....	733·7	654·1	469·5	21	--	—	—	—
3648		452·3	438·6	439·7	15	--	—	—	—
3659	II. Low P............	770·6	676·2	577·3	22	817·6	—	—	—
3643	IIIA. Low Ca..........	654·4	583·3	505·4	19	771·7	703 2	652·6	24
3655		542·7	505·0	458·2	17	798·1	845·7	790·1	27
3640	IIIB. Low Ca..........	-	—	-	—	672·9	738·4	661·7	23
3650	Low Mg..	621·0	682·6	526·2	20	858·1	766·9	736·1	26
3642	IV. All min. low except	714·8	419·1	240 7	15	719·7	692·0	617·3	22
3649	Ca and P.	663·5	470·3	254 1	15	730·9	660·4	659·6	23
3651	V. Low NaCl.........	554 4	426·5	306·9	14	--	—	—	---
3646		684·4	669·6	556 3	21	—	—	—	—
3658	VI. Low Cl	703·2	649·7	492·1	20	487·8	.	—	.
3675		715·5	557 3	483·0	19	--	—	—	—
3653	VII Low Na	1010·9	645·3	470·8	22	933·8	929·2	853·7	30
3656	VIII. Low K	—	—	—	---	—	—	- -	--
3673		536·1	492 9	271·9	14	---	—	- .	--
3677	IX. All min. sufficiency	465·4	634 6	482·2	16	601·4	413·0	488·0	17
3652	+ KI	512 5	428·8	382·1	15	—	—	—	—
3645	X. All min. sufficiency..	500 4	498·5	447·3	16	746·1	698·7	611 2	23

(*a*) NA, K, CL AND MG.

Omitting calcium and phosphorus for the time being and magnesium entirely, as it seems very improbable that the effects of "low" magnesium were felt at any time, sodium, chlorine and potassium are left to be dealt with. Undoubtedly a deficiency of sodium and of chlorine existed during the first part of the first lactation period. The result invariably was that milk production decreased until the quantity of the mineral secreted in the milk was definitely less than that contained in the food, although usually not much less. During the second lactation period the intake was always more than the milk content of any one of these three constituents, with the result that the lactating animals which lasted the full period, actually showed improvement in condition, or as in the case of the K low animal, which was in excellent condition at calving, showed no abnormal effect.

If Experiments V, VI, VIII and IX be reviewed, several points stand out which are worth recording: Animals Nos. 3642 and 3649, on a ration containing about 30 gm. K_2O, 5·8 gm. Na_2O, 7·3 gm. Cl for 20 months which included two gestation periods and one lactation, remained in very poor condition throughout the experiment and could easily be selected at any stage as the poorest group. The combined effect of low Na, K and Cl in the ration affected the animals adversely and resulted in permanent loss of condition and poor appetite. During the second lactation, when these animals never produced less than 2 gallons milk daily, they actually improved in condition and gained in weight on a ration containing 30 gm. K_2O, 15·5 Na_2O, 13·8 gm. Cl, while the secretion of these minerals in their milk was as follows: K_2O 24 gm.; Na_2O 7 gm.; Cl 10 gm. Animal No. 3653 on a sodium low ration fared similarly. 5·8 gm. Na_2O during the first 2 months of the experiment with its two gestation and one 90-day lactation periods, was apparently insufficient to meet the demands of the animal, but when this quantity was increased to 15·5 gm. Na_2O during second lactation, the animal easily stood the strain of producing over 3 gallons of milk daily. It must be noted that the daily milk contained 9·6 gm. Na_2O. In other words, No. 3652 secreted two-thirds of the total intake of sodium in her milk for 90 days without apparent ill effects upon her system. Cow No. 3673 receiving daily about 30 gm. K_2O during the first 20 months of the experiment with two gestation periods, one lactation period, during 50 days of which this animal suffered severely from mastitis which was followed by a long rest period, apparently did not suffer from a potassium deficiency. During second lactation, when this animal gave over 2 gallons of milk daily, containing 22 gm. K_2O, she remained in good condition throughout, and was apparently not abnormally affected by lactation. The conclusion seems justified that No. 3673 required during growth and pregnancy less than 30 gm. K_2O daily, while during a 90-day lactation period of over 2 gallons daily, 38 gm. K_2O were ample in spite of 22 gm. K_2O actually being secreted in the milk. Unfortunately no further light is thrown on the rôle of chlorine, as the remaining animal in the experiment after the first lactation period did not become pregnant. Tentatively it may be stated, in the absence of further data, that 7 gm. chlorine in the daily ration appears to have been on the low side during first gestation, when the animals were still actively growing, was definitely deficient during lactation, secretion in milk being greater than intake, but cannot be said to have had further ill effects. Judging from the effects of low sodium in the ration, one would be inclined to believe that during lactation at least one and a third times the amount of chlorine contained in the milk should be supplied in the food. Passing to the animals Nos. 3646 and 3651 on low sodium chloride it is perhaps strange that these animals did well on the whole in spite of having in addition to low sodium, with its detrimental effects upon No. 3653, also low chlorine. The only difference between the rations of No. 3653 on the one hand and Nos. 3646 and 3651 on the other is, that the ration of No. 3653 was potentially more acid, due to the presence of chlorine. The additional potential acidity was equivalent to 1620 c.c. normal solution, but from the available data it is impossible to judge this effect at all correctly. It can only be said that low sodium was detrimental,

whereas low sodium in addition to low chlorine was not; and thirdly that low sodium, low chlorine, low potassium again produced detrimental effects. It appears that the relation of these three constituents to one another is important and need further consideration.

Applying the information gained in these experiments with regard to low K, Na and chlorine in a ration to possible deficiencies in practice the following points are brought out:—

(1) Potassium deficiency, which cannot be said to have been brought about in Experiment VIII will probably never be present in two-gallon capacity lactating cows on pasture. Even ·4 per cent. K_2O in pasture, which is much lower than the usual figure, would supply about twice as much potassium as that contained in the potassium low ration.

(2) The chlorine content of the rations in Experiments V and VI was much lower than the intake of cows on very poor pasture (·15 per cent. chlorine). As a matter of fact, a 1,000 lb. cow on such pasture would probably ingest about 3 times as much chlorine as the animals in the chlorine low rations of this investigation.

(3) The sodium intake of the animals in Experiment VII on low sodium, was not particularly low. Mature South African pastures often contain as little as ·015 per cent. Na_2O under which conditions a 1,000 lb. cow would ingest only about as much sodium as that contained in the sodium low ration or, in other words, only about as much as she actually required for the production of 2 gallons of milk. Sodium deficiency in South African pastures at certain times of the year, when only fully grown mature and often old grass is available, is well worth the serious attention of investigators in this field.

(4) The combined effect of low Na, Cl and K was detrimental to the health of the animals, but there is little danger of procuring such an extremely low intake of these three constituents in animals on natural grazing.

(5) It is tentatively suggested that two-gallon lactating cows require daily, as a minimum, one and a half times the quantities of easily available Na and Cl secreted in the milk, i.e. about 15 gm. Na_2O and about 13 gm. Cl, while an intake of 38 gm. K_2O is sufficient to provide for the secretion of over 21 gm. K_2O in the milk without deleterious effects upon the cow.

(b) CALCIUM AND PHOSPHORUS.

With regard to Experiments I, II, III and IV on the rôle of P and Ca, the position is more clear. The often observed fact that low phosphorus in a ration brings about stywesiekte, poor condition, low inorganic phosphorus in the blood, and general unthriftiness, has been confirmed both in Experiment I and II. The latter experiment was a straightforward P deficiency, while the animals in the

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former were subject to low calcium in addition to low phosphorus. A consideration of Experiments III and IV indicates that the animals in both experiments withstood the low calcium quite well. On the other hand, Table III indicates that calcium deficiency was acute during both lactation periods. The animals, as already stated, ingested a certain amount of soil, so that the intake of calcium was probably higher than that given in Table II. However, apparently Ca deficiency was practically without effect upon the animals and the only possible conclusion seems to be that the intake of Ca with the soil must have increased the total intake of Ca very considerably, as it seems very improbable that the animals in Experiment III on low Ca could have continued to secrete in their milk, without more marked effect on their bodies, about twice the quantity of calcium that they were getting in their food. Clinical symptoms of calcium deficiency were not noticed in this investigation, and it is feared that reliable conclusions about the calcium requirements of cattle cannot be drawn until additional data have been obtained from an experiment now under consideration on the rôle of the Ca, P complex in the nutrition of bovines.

As suggested by a study of the phosphorus intake and outgo of Nos. 3641, 3648 and 3659 in Tables III and IV, by clinical symptoms of aphosphorosis in the course of the experiment, and by low inorganic phosphorus in the blood, phosphorus deficiency was acute for the greater part of the experiment. Expressed as P_2O_5 the demand for phosphorus during lactation is greater than that of any other mineral, and unfortunately, varies greatly from season to season.

It may be pointed out here that Crichton (1931) considers that one of the authors (A. I. M.) is of the opinion that inorganic phosphorus in the blood limits milk production, but that he erroneously attributed that view to the author concerned. It has been pointed out time and again from this Institute, that phosphorus deficiency limits milk production and is also *associated with* low inorganic phosphorus in the blood. But it certainly would be illogical and purely speculative for anyone to conclude that therefore inorganic phosphorus in the blood limits production. Under South African conditions, at the best of times, pasture will not usually contain more than ·45 per cent. P_2O_5 for any length of time. In such circumstances 25 lb. pasture (on dry basis) would contain about 50 gm. P_2O_5, which would hardly meet the requirements of a 2-gallon cow. It may safely be said that without drastic pasture improvement there is little hope of getting a daily milk production for any length of time of 2 gallons from cows on pasture only. Unfortunately low protein in such pasture would be an additional limiting factor for the production of 2 gallons milk. Phosphorus supply is undoubtedly a problem that has to be contended with under systems of milk production under ranching conditions.

(c) REPRODUCTION.

Before concluding, it is necessary to review reproduction in this investigation. In Table VI below the calving chart of the animals is given.

TABLE VI.

	First Calving, 1931.						Second Calving, 1932.				
D.O.B. of Cow.	D.O.B. of Calf.	Experiment.	Gestation Period. Days.	Birth Wt. of Calf.	Remarks Calves.	Remarks Cows.	D.O.B. of Calf.	Gestation Period. Days.	Birth Wt. of Calf.	Remarks Calves.	Remarks Cows.
3641	4543	Low Ca and P.	272	50	Normal..........	—	5212	284	78	Normal.	23.9.32 killed in extremis.
3648	4540		275	68	Normal..........	—	5265	281	87	Normal.	1.3.33 died sequel to shock to fall.
3659	4552	Low P....	279	63	Blind and strong	—	5207	282	87	Blind but strong	4.10.32 died of P deficiency.
3643	4535	Low Ca... High Mg..	267	65	Blind and weak..	—	5236	278	78	Normal	23.12.32 dismissed.
3655	4533		265	50	Normal..........	—	5209	280	90	Normal	7.12.32 killed for skeleton.
3640	—	Low Ca and Mg.	266	53	*Lived few hours, blind	—	5206	281	92	Normal	6.12.32 killed for skeleton.
3650	4536		277	45	Blind..........	—	5214	284	88	Normal	5.12.32 dismissed.
3642	4548	All min. def. except Ca and P.	268	67	Unable to get up	—	5203	283	64	Normal	— collected skeleton.
3649	4529	Low Na and Cl.	264	46	Blind and weak..	—	5208	278	81	Normal	26.11.32 dismissed.
3651	4541		268	68	Unable to get up	—	—	—	—	—	sterile, dismissed.
3646	4531		273	44	Weak, died 1 day old	—	—	—	—	—	8.12.31 died.
3658	4547	Low Cl....	277	63	Blind..........	—	5240	274	89	—	19.8.30 killed o/a T.B.
3675	4528		270	69	Unable to get up	—	—	—	—	—	29.10.32 died of peritonitis.
3653	4534	Low Na...	271	58	Unable to get up	—	5204	283	76	Normal	20.11.32 dismissed.
3673	4546	Low K....	280	50	Blind..........	—	5296	284	78	Normal	6.5.33 dismissed, peritonitis.
3656	—		262	46	*Aborted..........	—	—	—	—	—	died, peritonitis.
3677	4538	All min. suf- ficiency, plus KI	272	67	Normal..........	—	5232	280	82	Normal	— dismissed, T.B.
3652	4539		273	61	Blind..........	—	5226 5227	273	51 65	Normal Normal	—
3645	—	All min. suf- ficiency	263	55	*Aborted, normally developed	—	5237	284	100	Normal	27.12.32 dismissed.

A glance at Table VI reveals the fact that in 1931 calving was abnormal. Weak and blind calves were the rule, and almost 50 per cent. of the calf crop was blind. The vitality of the calves was low in practically all cases. It seems that a factor present in all the experiments was responsible for poor reproduction, and it is for this reason that 5 lb. ensilage were added to the basal ration of each animal in 1932, i.e. about 6 months before second calving period was due. The increase of minerals was negligible as shown in Table II, but subsequent calving was a complete success.

In the light of this work and of that of Hart, Hadley and Humphrey (1932), where similar results were obtained with a basal ration consisting primarily of wheat products, it appears that the birth of abnormal calves in this experiment should be associated with an inherent deficiency in the ration, such as poor quality protein or perhaps vitamin A. 5 lb. of ensilage containing 80 per cent. water, can hardly be said to add much " quantity " of any food constituents to the ration, while it is rich in vitamins, as is all green feed. The problem of poor reproduction must be left unsolved until the experiment now being conducted to elucidate this point has yielded some information, and it is taken for granted provisionally, especially when the two calving periods are compared, that abnormal calving in 1931 was not associated with any specific factor related to the mineral composition of the rations. Apparently the 5 lb. ensilage which, according to Table II, hardly increased the mineral content of the basal ration at all, contained the factor or factors which made calving in 1932 one hundred per cent. successful. No weak or abnormal calves were born, although the number of animals still alive to calve down was considerably less than in 1931.

VI. SUMMARY.

1. Data are presented on the requirements of growing and lactating cattle of Ca, P, Mg, Na, K, Cl and I.

2. The investigation lasted approximately $2\frac{1}{2}$ years; the animals passed through 2 gestation periods and two lactation periods of 90 days each.

3. A pair of grade Friesland heifers was placed in each experiment on a basal ration of $3\frac{1}{2}$ lb. hay, 10 lb. maize concentrates [crushed maize and/or maize endosperm (fanko)] and 20 gm. blood meal.

4. The mineral content of the basal ration was as follows: CaO 6·8 gm.; MgO 11·1 gm.; K_2O 29·3 gm.; Na_2O 5·7 gm.; P_2O_5 15·4 gm. and when crushed maize was eliminated 8·4 gm.; Cl 6·7 gm. and SO_3 12·9 gm. All the minerals were given as supplements daily, except Sundays, with the concentrates.

5. The animals were inspected daily for symptoms of deficiency, weights were registered monthly, food consumption checked and samples of blood drawn monthly for the determination of their mineral content.

6. The animals were kept over night under roof in feeding boxes in an open shed. During the day they were allowed to exercise in a paddock with concrete floor, except on some occasions when they were allowed into an adjoining paddock with a surface of sand.

7. 0·1 gm. potassium iodide had no visible effect upon the pair of bovines in this experiment.

8. It is provisionally suggested that 14 gm. Cl was enough to provide for the daily secretion of 2 gallons of milk and that this amount would also be enough for growth and gestation.

9. When one and a half times the quantity of sodium contained in 2 gallons of milk is supplied in the food, this is apparently enough for the normal production of such a quantity of milk, i.e. about 15 gm. Na_2O per day.

10. ·38 gm. of K_2O was enough for the production of 2 gallons of milk daily for 90 days without ill effects.

11. There is very little possibility of a K or a Cl deficiency when 2-gallon cows are run on pasture only.

12. The sodium content of a 1,000 lb. cow's ration of South African pasture ·015 per cent. Na_2O is often below that of the basal ration, which is deficient for the production of 2 gallons of milk daily.

13. It was impossible to produce clinical or other symptoms of acalcicosis. An explanation is offered tentatively.

14. Aphosphorosis—incipient and clinical—was easily produced on a ration both low and not so low in phosphorus.

15. Radiographs of selected bones of phosphorus and calcium deficient animals are presented.

16. A lactating animal's defence against mineral deficiency is decrease in milk production, the greatest decrease being noticeable when the deficiency is greatest, e.g. Experiment IV, low K, Na and Cl.

17. It is doubted whether low magnesium could possibly become a problem in an animal's diet. The basal diet composed of materials low in minerals contained at least 5 times the amount of magnesium secreted in 2 gallons milk.

18. The present study is being followed up by a study of the rôle of the Ca, P complex in the nutrition of cattle and by further studies on Na, K and chlorine low rations.

REFERENCES.

BECKA, J. (1929). *Prager Archiv. für Tiermedizin* Teil A. Heft 1, 2 and 3, pp. 253-259.

CRICHTON, J. A. (1930). *Jnl. Dairy Research.* Vol. II, No. 1.

DU TOIT, P. J., MALAN, A. I., GROENEWALD, J. W., AND DE KOCK, G. (1932). Studies in Mineral Metabolism XXVI. The effect of fluorine on pregnant heifers. 18*th Rept. Dir. Vety. Serv. and Anim. Indust.* Union of S.A., pp. 805-817.

GROENEWALD, J. W. (1934). The influence of rations low in certain minerals on the composition of the blood and milk of cows and on the blood of their progeny. Later issue of this *Journal.*

HART, E. B., HADLEY, F. B., AND HUMPHREY, G. C. (1932). The relation of nutrition to contagious cattle abortion. *Research Bulletin* 112. Agric. Expt. Stn. University of Wisconsin, Madison.

MAREK, J. (1924). *Archiv. fur Tierheilkunde.* Bd. 51, Heft 1, pp. 1-14.

MAREK, J., AND WELMANN, O. (1932). Two volumes. Publishers G. Fischer. Jena.

REED, O. E., AND HUFFMAN, C. F. (1930). The results of a five-year mineral feeding investigation with dairy cattle. *Mich. Agric. Exp. Stn. Tech. Bul.,* 105.

SJOLLEMA, B. (1932). Onderzoekingen over de oorzaken van grastetanie. *Tijdsch. voor Diergeneesk.,* 59e Deel. Also Vol. 57, pp. 1229-1244.

THEILER, A., GREEN, H. H., AND DU TOIT, P. J. (1927). Minimum mineral requirements in cattle. *Jnl. Agric. Science,* Vol. 17, pp. 291-314.

WELMANN (1931). *Nutrition abs. and Reviews.* Vol. 1, Nos. 1 and 2, pp. 241-242.

A Study of the Mineral Content and Feeding Value of Natural Pastures in the Union of South Africa.

(SECOND REPORT.)

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CONTENTS.

INTRODUCTION.

MENTION is made under " outline of investigation " in the first report (1932) on the problem stated above, of experimental grass plots forming a part of the experimental plan of the investigation. It is proposed to deal with the results of the first twelve months' work upon the experimental plots in this publication. Eleven species of grasses were subjected to the treatment according to the experimental plan, for the full period, as the analyses indicate.

The objects of the plot experiments may be restated here briefly : —

(1) A study of the effect of growth on the chemical composition of pasture. Monthly, two-monthly, three-monthly, etc., cuttings of each variety of grass were made and analysed.

(2) A study of the variation in chemical composition of different species of grasses at the same period of growth.

(3) The effect of seasonal variation in growth on the chemical composition and on the yield of grass.

(4) A study of the chemical composition of successive cuttings of the same variety of grass. This means that, if the work began in January, the analysis of the monthly growth during each month of the year would be available. The analyses of the two-monthly growth of the same portion of the plot would be made every alternate month, for three months' growth every three months and so on, so that the 12 months' growth would be ready for analysis at the end of the year.

(5) The determination of the chemical composition of different parts of the same plant* at different stages of growth. For this purpose leaves, stalks, haulms, were analysed separately at the different periods of growth.

DETAILS OF PLOT EXPERIMENT.

In August, 1931, this aspect of the work was begun by planting eleven species of grasses in separate plots, 30 by 14 feet, mapped out in an area where indefinite extension was possible and where the soil was apparently uniform in composition over the whole area. The latter point was confirmed subsequently by chemical analyses of soil samples from each plot. Other plots have been planted from time to time, so that at present more than three times the original number of grass species are included in the work. However, this report deals only with the eleven species already referred to which were planted at the outset, and of which the analyses have been carried out for the first 12 months of the experimental period, i.e. from February, 1932, until January, 1933. The same method of establishing the plots has been followed throughout and will be described in detail.

The soil was dug up once, all vegetable matter removed, the ground harrowed, the plots mapped out so that each was surrounded by a path 3½ feet wide, and after the first good rain in August the grasses planted in rows 10 inches apart. After that the plots were watered until the grasses had established themselves, when they were given no further attention except being weeded when necessary. Some grasses did not grow as easily as others, and portions of the plots had to be replanted. However, all eleven plots were established successfully before the end of 1931. They were then left to grow freely until the end of January, 1932, when the investigations into their chemical composition was begun, and the grasses received no further attention, except occasional weeding, as already stated.

The grasses on all the plots were cut short—about half an inch from the ground—on the 27th January, and the following plan of experiment followed. On the 27th of each month from February, 1932, until January, 1933, samples were taken from each plot. The first row of grass on each plot was cut monthly and therefore yielded 12 samples of monthly growth of the grass in question. The second row was cut every two months, i.e. on the 27th of March, May, July, etc., and therefore yielded six samples of two months' growth each. The third row was cut every three months, the fourth every four, and so on, until the last or twelfth row was cut on the 27th January, 1933, after twelve months' growth. Each of the samples so obtained was analysed for phosphorus, calcium, magnesium, potassium, sodium, chlorine, protein and fibre.

It is realized, of course, that the plots are much too small to study yield, nor was it our intention to do so, although the differences in the weights of the grasses cut in the various rows uniformly planted would serve as an index of the amount of growth of that particular grass during the period in question. In other words, the samples were weighed, merely to derive at a figure suggesting growth during the period, and not to compare carefully the production of grass of one species with that of another species for the same period.

Other observations in regard to rate of growth, effect of cutting at different times, method of growth at different seasons, resistance against drought, response to climatic conditions, were made from time to time on the plots studied, and will be considered in the discussion of the results at a later stage in this article.

As the prevailing climatic conditions were undoubtedly the determining factors in regard to the growth of the grasses, these are given as fully as possible in Table I, to which reference will be made frequently.

TABLE I.

1932.		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
February....	Rainfall	—	0 66	0·10	1·05	0·30	—	—	—	—	—	—	—	—
	R. humidity	64	65	75	76	88	67	77	68	69	67	66	72	65
	Sunshine (hrs.)	9·3	11·0	10·3	8·3	8 0	7 3	6·0	7·0	10·3	11·0	5·4	8·0	9·3
	Max. Temp	93 0	89 0	85·0	85·0	82·6	85·0	82·0	83·4	84·0	88·0	84·4	86·4	81·6
	Min. Temp	57·0	60·0	60 0	63·8	58 0	60·0	65 0	63·0	57·0	58·4	66·0	63·0	64·0
March	Rainfall	—	—	—	—	—	—	—	0·07	1·58	—	—	0·05	—
	R. humidity	77	58	70	80	75	59	66	68	75	87	49	62	92
	Sunshine (hrs.)	3·3	10 3	10·1	9·15	10·3	9 2	10·2	7 45	2·30	4·45	8·0	9·4	1·3
	Max. Temp	79·0	89·8	87·4	81 4	86 0	85·0	85·0	90 8	85·0	81·6	89·6	80·6	69 4
	Min. Temp	64 8	56	59	61	59 4	57	53	59	67	63·6	59·6	59	60 8
April	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	63	60	68	66	63	63	63	53	68	63	57	65	58
	Sunshine (hrs.)	10·1	10 2	9 3	10·3	10 0	10·0	10·0	10 0	9 2	9·0	9·0	10·0	10·1
	Max. Temp	85	85	87	85	85	81·8	83·2	80 4	83·4	84·8	85	82·4	85
	Min. Temp	53	46	49	49	48	48	45·6	49	56	46	49·6	50	43
May	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	0·10
	R. humidity	65	69	62	65	70	72	56	51	62	60	59	65	58
	Sunshine (hrs.)	9·0	9·0	6·3	8·1	8·1	9·0	9·3	9 3	8·3	9 0	7·0	9·0	9 2
	Max. Temp	73 6	69	69·4	71·8	74·2	74	78 4	77·4	76	78	77 8	83	84
	Min. Temp	49	56	49	49	40·4	38·0	38	39	38	37 6	38·6	39	43
June	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	68	76	76	68	58	71	65	68	58	57	45	48	42
	Sunshine (hrs.)	9 2	0·15	1·3	7 3	8 4	8 2	8 4	9·0	9 0	9 1	9·1	9·0	9·2
	Max. Temp	77	65	68·8	75 4	76·2	73 2	73·8	57·8	68	72	74	77	78
	Min. Temp	38·4	39	51	36	42 4	35 0	31 0	35·0	39·4	34	34	30	32
July	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	54	53	55	58	51	57	60	64	71	54	49	41	39
	Sunshine (hrs)	9·1	9·2	9·0	9·1	9·2	5 3	9·1	9·1	9·0	8·4	9 2	9 1	9 1
	Max. Temp	74	77	73	77	76	76	58	64	66	70	75	74	73
	Min. Temp	30	31	30	32	32	34	38	32	29	27	29	29	27
August.	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	44	50	38	62	44	39	39	39	34	38	50	44	35
	Sunshine (hrs).	9·2	9 1	9·3	9·3	9 3	9·4	9·4	9 5	9·4	9 4	9 4	9 5	9·4
	Max. Temp.	74	77	72	71	75	75	77	76	79	75	75	76	78
	Min. Temp	31	32	34	31	32	31	30	32	35	32	30	30	32
September...	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	—	51	60	61	41	56	39	43	47	32	19	—	53
	Sunshine (hrs.)	—	10 0	10·1	10 1	10 7	10·1	6·3	7·3	10 0	10 3	—	—	8·1
	Max. Temp.	87	85	79	84	86	79	81	78	77	83	—	83	76
	Min. Temp.	—	42	46	42	41	45	46	51	43	35	41	—	40
October....	Rainfall	0 12	—	0·88	0·58	—	—	—	—	—	—	—	—	—
	R. humidity	72	77	27	81	71	78	66	50	31	60	54	39	55
	Sunshine (hrs.)	3·0	8 0	7·3	0·3	3·1	5·0	8·3	10·2	10 3	10·1	10 3	10·3	10 3
	Max. Temp.	78	63	86	72	74	74	84	94	97	90	93	88	84
	Min. Temp.	54	56	56	56	57	49	50	57	59	60	61	60	57
November	Rainfall	0·03	—	—	0·06	—	—	0·72	—	—	0 22	—	—	—
	R. humidity	31	48	47	40	55	18	59	81	48	58	73	75	66
	Sunshine (hrs.)	10 2	10·3	5 0	8·0	11·0	10 4	9 3	5 1	10 4	7·3	7 4	4·3	10·3
	Max. Temp	81	87	77	75	78	92	74	74	76	76	75	76	75
	Min. Temp	54	58	57	59	52	60	61	58	59	55	52	57	57
December....	Rainfall	—	—	—	0·5	—	—	—	—	—	—	—	0·06	0·09
	R. humidity	90	76	78	68	57	75	68	61	60	67	35	62	71
	Sunshine (hrs.)	6·0	6·1	6·4	1·1	6·4	0·5	9 1	5·3	11·4	10 3	11·4	11·0	9·4
	Max. Temp	81	87	87	75	79	77	93	89	93	93	97	97	95
	Min. Temp	63	63	65	59	60	55	59	63	60	61	53	58	63
1933. January.....	Rainfall	—	—	—	—	—	—	—	—	—	—	—	—	—
	R. humidity	61	60	56	50	65	60	44	70	64	50	60	45	66
	Sunshine (hrs.)	7·4	9·3	8·2	11·4	9 0	10·4	11·4	6·4	10·5	5·4	5·1	8·0	6·2
	Max. Temp.	83	90	90	95	89	94	97	89	93	91	89	89	93
	Min. Temp.	54	55	61	60	66	60	58	67	62	63	59	61	65

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METEOROLOGICAL DATA.

14th	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th	25th	26th	27th	28th	29th	30th	31st
0·16	—	—	1·4	—	0·43	0·05	—	—	—	—	0 20	—	—	0·48	0·02	—	..
57	67	68	66	73	78	48	70	68	—	58	52	78	71	73	90	—	..
7·4	10·0	10 2	6·0	10 0	2·3	7 4	9·1	9·0	6·4	10 3	8·1	3·0	9 1	2·4	3·1	—	..
80·0	89·8	90·4	83·6	85 0	79·6	86 5	78·0	81·2	91 0	97·0	90 2	85·0	83 5	82 0	82 8	—	—
—	60·0	59·0	62 0	61 0	64 0	62 0	55 0	57·0	60 0	57 0	64·0	62·0	64 0	61·0	61·0	—	—
—	—	—	—	—	—	0·29	0·05	—	—	—	—	—	—	—	0·04	—	—
64	78	58	73	81	70	86	73	75	75	64	63	70	57	66	67	64	66
10 2	4 4	2 0	2·3	5 3	4·3	2·4	9 1	5 4	8 0	10·0	7 3	9·0	8·0	7 2	6·2	8 3	10·0
78 2	84·0	83·0	78 6	80 0	83·8	78·0	80·6	79·0	83 8	85 0	82 6	87 2	90 2	89·0	85·0	86·6	85·4
51	50	60	62	57	63	62	53	60	51	53	53	54	60	55	57	53·0	54 0
—	—	—	—	—	—	—	—	—	0·17	0·23	0 04	0 31	—	—	—	—	—
51	63	54	44	42	45	45	49	63	73	95	95	95	79	78	68	65	—
10·1	10 0	10 0	9 4	10 2	10 1	7 3	7 0	2 0	0 0	0 0	3 0	5·1	6·4	9 4	8·0	9·0	—
85	84	86 2	89	90	90	90	91	72 2	74 0	65	73 6	78·6	76	77·4	80	79	—
42	44	43	47	51	45	50·8	53	51	52	57	56	52	55	51	49	52	—
—	0 05	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
93	95	69	74	66	68	61	55	59	47	53	59	55	54	60	73	73	59
3 4	8 0	7 2	4 4	9·1	9 0	9 0	9·0	5 3	9 1	9·1	9·2	9 2	9 2	9·1	9 0	9 1	9 0
68 8	68	66 6	69 4	70	73	74	76	77	75	77	76·4	78	81	79	73	77	80
54	47·8	45·6	44 8	33 2	31 8	30 4	33	42	38	36	34	33 8	36·6	39	42 2	39	41 6
45	53	47	39	47	63	39	54	51	51	51	57	78	54	29	50	50	—
9 3	9·1	9 1	9 2	9·1	9·0	9 3	9 1	9·0	9 2	9 0	9 0	9 0	9 3	9·2	9 2	9·2	—
77	75	76	72	74	75	73	74	73	74	71	68	71	74	74	76	75	—
32	32	29	34	32	36	28	31	32	30	44	29	28	29	36	38	32	—
54	65	31	51	71	86	48	50	45	31	49	32	37	45	29	29	41	35
9 1	9·2	8 4	9 0	9 1	9 0	9 1	9 2	9·0	9·1	0 0	9 3	9 1	9 2	9 3	9 3	9 3	9·0
68	65	67	65	68	70	72	64	67	74	73	76	72	74	73	73	75	75
36	28	25	35	28	30	32	33	26	26	29	30	28	28	32	27	30·4	36
30	60	62	61	57	40	21	33	29	24	24	73	58	35	32	28	58	41
9 3	9 4	0·5	10·0	9 4	10 0	10 0	9 4	10 1	10·1	10 0	9 1	9 4	10 1	10 0	10 0	10 0	10 0
78	74	71	70	74	77	82	83	81	77	75	79	84	85	84	83	83	—
33	50	42	34	31	34	37	36	38	39	42	41	38	39	41	36	41	42
—	—	—	—	—	—	0 87	—	0 01	—	—	—	—	—	—	—	—	—
33	27	44	67	44	43	71	76	57	70	53	35	8	16	53	24	48	—
10·3	10 5	6 3	6 3	10·0	9 4	8 3	10 1	9 0	3 0	7 0	8 5	10·0	10 2	10 2	7 4	9 3	—
82	89	78	78	77	80	78	81	86	76	83	76	77	90	90	85	83	—
43	42	58	62	52	56	56	50	50	62	55	51	45	48	50	56	54	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·18	—	—	—
45	31	31	31	55	48	35	57	60	51	48	35	25	63	48	65	76	45
10 4	10 2	7 4	10·3	10·0	10 3	10 0	8 0	10 4	8 4	8 4	5 0	10 4	9 3	7·5	3 5	6·0	8 5
88	89	93	97	84	90	92	81	76	81	84	82	93	84	76	70	75	77
62	52	53	55	65	54	57	65	49	49	59	58	55	61	57	55	52	45
—	—	—	—	—	0 28	0 18	—	0 40	0 04	—	0 24	—	—	—	0 37	—	—
55	61	57	39	43	44	73	70	59	73	63	63	76	28	62	65	—	—
9·4	10 3	8·4	9·3	10 1	8 3	3 0	3 0	8·4	8·4	11·1	2 4	11·3	10 4	11 3	10·1	9·4	—
74	76	79	76	80	74	75	75	75	75	91	88	90	93	95	96	96	—
58	59	61	60	60	65	61	61	61	—	59	68	56	59	64	64	64	—
0·56	—	0·35	—	—	—	0·12	—	—	0·54	—	1 3	—	—	—	0 02	—	—
48	58	70	73	79	—	61	64	67	64	48	66	73	93	67	63	57	76
7·3	7 4	6 0	9 1	11·3	9·3	10 1	10·0	9 4	11 0	3 2	4 1	1·1	10 0	10·3	12·0	2·1	1 0
04	92	93	89	87	90	87	90	92	93	83	87	77	82	80	83	81	75
59	63	65	58	64	62	63	61	61	63	64	64	63	60	59	54	59	60
0·21	1·06	0 13	0·03	0 08	—	0 03	—	—	—	—	—	—	—	—	—	—	—
66	86	86	90	79	79	74	73	57	61	35	51	50	57	63	50	56	65
10·0	8·1	0·0	1·0	4 0	4·3	3·3	10 2	9·4	11·0	11·2	11·2	11·1	11·1	10·3	10 4	11·0	8·5
88	90	75	79	81	84	86	92	86	86	88	94	91	90	99	88	86	89
64	59	63	62	65	67	66	60	66	64	55	55	57	58	57	60	60	57

Several important factors are noticed in Table I which bear more or less directly on the subject of plant growth:—

(a) The total rainfall for the twelve months, 1st February, 1932, to 31st January, 1933, was 17·43 inches, compared with the normal average of 29 inches per annum. The unequal distribution of rain over the year is also worthy of note.

(b) No rain fell during the months June, July, August, and almost none in May.

(c) The first rain in spring fell on the 20th of September, so that plant growth after winter and up to that time was very little indeed, if any at all.

(d) With abundant sunshine and low relative humidity of the air, the rain could not be used to the greatest advantage by the plants. Such conditions would favour the rapid evaporation of soil moisture, and one would anticipate short periods of rapid growth immediately after a good rain followed by poorer growth or complete cessation of growth according to amount of soil moisture present until more precipitation takes place, when growth would begin again. This point will be referred to again in the article.

(e) The grasses were grown essentially under conditions of summer rainfall, when practically all the growth took place. During the 1932 winter, growth almost ceased on account of drought and cold.

(f) The effect of the climatic conditions on the composition of the grasses cannot be gauged correctly until data for subsequent years have become available. At the same time it must be pointed out that all the grasses under consideration were subject to the same climatic conditions, and the results, therefore, are on a comparative basis.

METHODS OF ANALYSIS.

The methods employed in the analytical work were described fully in the first article. A few modifications that have since been introduced will be referred to briefly here: A new washing mixture in which caustic soda was replaced by ammonia was made up for magnesium determinations, and gave better results than that described by Malan and Van der Lingen (1930). For greater detail of the method reference must be made to an article by one of the authors (C.R.H.) in this Journal. The procedure for the determination of sodium was modified slightly and proved to be an improvement in every way on the original method. The principle of the method remained unaltered, and details are to be found in an article by J. G. Louw (1933). The absence of total ash determination remains a difficulty, and as the results now stand, the undetermined fraction consists of N-free extract, either soluble extract and ash, causing the first and most important fraction, viz., N-free extract to be only approximate. Part of the ash is, of course, that actually determined and given as mineral constituents. Still, the silica content is not known nor can mechanical contaminations with sand during the

process of sampling and classifying be excluded entirely or judged accurately while the extent of these factors undoubtedly affect the figure for N-free extractives.

Sand or soil contamination of the grass samples in the work under discussion was practically negligible, as the process of cutting is such, that would eliminate all contamination, and only mechanically adhering particles on the outside of the plant material might have found their way into the sampling bag. Briefly, the method of sampling is as follows: The superficial parts of the grass are grasped with the left hand, and cut about half an inch above the soil surface with a pair of scissors held in the right hand. The sample is then immediately transferred to the bag or container. The samples are then taken to a room and, when air dry, are again shaken and handled before a final sample is taken to be milled and analysed. The resulting sample for analysis would probably be as free from mechanically adhering sand particles as is practicable to get it under ordinary circumstances when several thousand complete analyses have to be carried out annually in routine procedure. Under the conditions described above the effect of the ultimately adhering soil particles on the values obtained for the chemical analyses of the pasture samples is to all intents and purposes negligible.

Unfortunately, however, the value for N-free extract is not entirely corrected by the procedure described, while the factors which are not protein, not fibre and not the inorganic constituents given in the list of determinations, remain undetermined, the percentage N-free extract obtained by difference between the percentage values obtained and 100 must remain approximate. As explained in the previous publication, the reason for omitting some determinations such as ether extract, silica free ash, etc., and therefore, including these automatically in the N-free extract fraction, was due to limitation of available assistance, while at the same time no serious error was introduced. Several of the mineral surveys, some of which have been dealt with in the previous article already referred to, and the analyses of the samples of the first 12 months of the plot experiments, have been discussed on this basis, while with more assistance, which has recently been forthcoming, a method for the determination of total ash and soluble ash in addition to the other fractions has been introduced automatically, thereby providing figures that are more truly accurate for the N-free extract. Full details of the ash determinations will be given in this Journal by J. G. Louw (1933).

PRESENTATION OF RESULTS.

As already stated, eleven species of grasses were studied and analysed, each giving a maximum of 12 monthly cuttings, 6 bi-monthly cuttings, 4 three-monthly cuttings, 3 four-monthly cuttings, 2 five-monthly cuttings, and 2 six-monthly cuttings, from February, 1932, to January, 1933. Actually, of course, growth did not take place equally well all the year round, so that none of the grasses gave the full number of monthly cuttings for the period in question. Nevertheless, six mineral constituents with protein and fibre, were regularly determined in each sample taken, involving several thousand analyses which present a problem as to the best method of presenting the results.

TABLE II.
P$_2$O$_5$ Content of Grasses.

(Period of growth: 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	·40	·31	·42	·43	·29	·45	·46	·53	·38	·40	·59
March	·29	·29	·30	·27	·21	·32	·28	·30	·24	·23	·30
April	·17	·20	·18	·22	·12	·19	·17	·14	·17	·12	·16
May	·14	·16	·15	·13	·08	·14	·13	·10	·13	·07	·18
June	·13	·11	·09	·10	·07	·12	·12	·09	·07	·06	·16
July	·12	·12	·09	·09	·06	·11	·11	·09	·05	·05	·16
August	·15	·10	·08	·07	·06	·12	·10	·08	·08	·06	·12
September	·11	·11	·11	·11	·06	·19	·11	·09	·10	·07	·25
October	—	·14	·12	·10	·06	·13	·14	·11	·13	·10	·18
November	·10	·13	·12	·12	·06	·21	·12	·10	·11	·10	·22
December	·11	·16	·13	·09	·09	·17	·13	·12	·11	·11	·18
1933.											
January	·15	·16	·12	·10	·10	·27	·13	·13	·11	·12	·22

It appears best to deal with each constituent determined separately after the tables of analyses for that constituent have been given. The constituents to be considered are phosphorus, calcium, magnesium, sodium, potassium, chlorine, protein, and fibre.

Two tables will be given to represent the results of the analyses in regard to each of the constituents to be considered. The first gives the percentage content of the grasses of the particular constituent at the following periods of growth: 1 month, 2 months, 3 months and so on, up to twelve months. The second table gives the analyses of the grasses every month, another portion of the plot cut every two months, a third cut every three months, and so on, up to the two six-monthly cuttings. In each table, therefore, data are presented for the full 12-month period, i.e. from 27th January, 1932, until 26th January, 1933.

<div align="center">PHOSPHORUS.</div>

Tables II and IV present in tabular form the data obtained in regard to the phosphorus content of the eleven species of grasses studied.

A close study of Table II is both interesting and essential. As reference to the meteorological data given in Table I will be made constantly, a summary of Table I is given below, which will present at a glance the general trend of the climatic conditions during the period to be considered, viz., February, 1932, until January, 1933.

<div align="center">

TABLE III.

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	Rainfall in Inches.	Dates on which Rain Fell.	Average Humidity.	Average Hours Sunshine.	Average Maximum Temp.	Average Minimum Temp.
1932.						
February.....	4·85	2. 3. 4, 5, 14, 17, 19, 20, 25, 28, 29	69	7·7	86	61
March........	2 08	8, 9, 12, 20, 21, 29	70	6·9	84	58
April........	0 77	23, 24, 25, 26..	64	8·1	82	49
May..........	0 15	13, 15.........	63	8 6	75	40
June.........	—	—	55	8·4	73	34
July.........	—	—	50	9·0	71	30
August.......	—	—	43	9 6	77	36
September....	0 88	20, 22.........	45	8·8	82	49
October.......	1 76	1, 3, 4, 28......	52	8 1	84	56
November....	2·54	1, 4, 7, 10, 19, 20, 22, 23, 25, 30	56	8 5	81	59
December.....	3·54	4, 12, 13, 14, 16, 20, 24, 26, 30	67	7 4	87	61
1933.						
January......	1·54	14, 15, 16, 17, 18, 20	61	8·2	89	61

For all the species of grasses given in Table II a rapid drop in the percentage prosphorus occurred until new growth in spring began, when a slight improvement set in which was apparently kept up until the end of the period under consideration. Reference to Table III indicates that the first rains after the dry winter months fell in September, so that the September cuttings already contained some new growth, as is also evident from the observations given in Table A in the appendix for September. Another striking feature of the values for P_2O_5 given in Table II is the extraordinary low phosphorus content of the grasses. The values for February, i.e. after the grasses had grown for one month, represent fair figures, although quite a remarkable variation is noticeable in the phosphorus contents of the species given, *Urochloa pullulans*, one of the best and most palatable grasses, being the highest, and *Hyparrhenia hirta*, a coarse, fibrous grass, hardly ever eaten and usually called thatch grass, being the lowest in phosphorus. After two months' growth, i.e. at the end of March, about thirty per cent. drop in phosphorus had already set in, while the values after that period represent those of mature grass with hardly any new growth as a closer study of the descriptions given in Table A in the appendix will reveal.

This table undoubtedly suggests that winter pasture, even if the best feeding grasses like *Urochloa pullulans*, *Panicum maximum*, etc., are present, does not contain more than about ·1 per cent. P_2O_5 if it consists of mature grasses as it usually does on account of certain pasture being especially reserved for winter grazing. Twenty-five lb. of such pasture—the daily requirements of an average cow—contain about 12 gm. P_2O_5, some of which is not available and will pass out in the faeces of the animal, whereas a gallon cow loses about 15 gm. P_2O_5 in her milk apart from that required for maintenance. The phosphorus content of this type of grazing is quite insufficient for even poor milk production during winter under ranching conditions where no supplementary feeding takes place, if the figures obtained on the plots are at all representative of the phosphorus content of the natural pasture.

In spite of fairly good rains having fallen in November and December, and well distributed over the month, the phosphorus content of the grasses did not show a significant improvement, although, according to Table A, quite a fair amount of new growth had taken place. The figures obtained for the phosphorus content after new growth had begun, do not claim to represent the phosphorus content of the grasses as eaten by an animal which would graze selectively, probably including more young grass and less old grass than a representative sample of the whole plot for analyses would contain.

Table IV gives the P_2O_5 content of the grasses under consideration after definite periods of growth at various times of the year. For instance, six cuttings of the same portion of the plot were made of two months' growth each from February, 1932, until January, 1933.

Of the species of grasses considered *Urochloa pullulans* retained its comparatively high phosphorus content, when the same portion of the plot was cut monthly, better than the others. Both *Cynodon dactylon* and *Hyparrhenia hirta* remained more constant in their

TABLE IV.

P_2O_5 CONTENT OF GRASSES.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodts.	Eragrostis superba.	Hyparrhenia hrta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	·40	·31	·42	·43	·29	·45	·46	·53	·40	·383	·59
March	·472	·336	·46	·47	·35	·494	·485	·565	·43	·44	·60
April	·37	·28	·43	·40	·31	·43	·43	·48	·38	—	·51
May	—	—	·47	—	—	—	—	—	·41	—	—
August	—	—	·415	·42	—	—	—	—	—	·39	—
September	—	·29	·34	·31	·28	·38	·40	—	·34	·30	·46
October	·27	·29	·306	·30	·28	·45	·35	·40	·32	·32	·50
November	·33	·28	·34	·25	·28	·44	·31	·44	·31	·29	·50
December	·31	·295	·32	·32	·32	·39	·36	—	·25	·30	·49
1933 January	—	—	·37	—	—	—	—	—	·31	—	—
Two-monthly Growth.											
1932 March	·29	·287	·30	·27	·21	·32	·28	·30	·23	·236	·296
May	·30	·18	·34	·31	·28	·34	·38	·35	·27	·29	·36
July	·38	—	·395	·47	·29	—	—	—	·33	—	—
September	·235	·23	·31	·18	·34	·526	·29	·38	·37	·32	·46
November	·24	·25	·22	·20	·22	·34	·26	·33	·23	·21	·42
1933 January	—	—	·24	—	·21	·27	—	—	·25	·23	—
Three-monthly Growth.											
1932 April	·17	·20	·18	·22	·12	·19	·17	·14	·12	·17	·16
July	·29	·127	·28	—	·25	·38	·42	—	·19	·19	—
October	·26	·265	·236	·28	·24	·28	·36	·46	·33	·22	·526
1933 January	·21	·226	·19	·20	·19	·25	·24	·32	·22	·19	·41
Four-monthly Growth.											
1932 May	·14	·16	·15	·13	·078	·14	·13	·10	·07	·13	·18
September	·33	·185	·295	·40	·34	·75	·47	—	·46	·27	1·12
1933 January	·20	·20	·19	·17	·21	·23	·20	·27	·21	·14	·41
Five-monthly Growth.											
1932 June	·13	·11	·09	·097	·072	·12	·12	·09	·056	·07	·16
November	·25	·20	·20	·16	·22	·32	·25	·37	·23	·16	·43
Six-monthly Growth.											
1932 July	·12	·116	·095	·089	·063	·11	·11	·086	·05	·054	·16
1933 January	·19	·19	·17	·17	·18	·24	·20	·28	·21	·15	·43

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phosphorus content, but the values remained low throughout. All the other species showed a greater or smaller drop in their values for phosphorus from February, 1932, to January, 1933. A study of the meteorological data given in Table III does not throw further light on this observation, and it must be left provisionally until the analyses for at least another year have been completed.

There was undoubtedly significant differences in the amount of growth during winter. In the case of *Cymbopogon plurinodes* only June and July did not provide a sample of monthly growth for analysis, while at the other extreme *Amphilophis insculpta* did not show enough growth for analysis of monthly cuttings from May to October.

The analyses of the remaining periodic cuttings all suggest the same thing, viz., that the older the plant, i.e. the later the stage of growth, the lower is its phosphorus content. Two-monthly cuttings show lower figures than one-monthly, while five- and six-monthly growth is definitely lower in phosphorus than three- and four-monthly growths.

The exceptions to this generalization should be studied in conjunction with Table III, and Tables A, B and C in the appendix. For instance, in Table IV *Eragrostis superba* and *Pennisetum ciliare* show a higher figure for phosphorus in September for a two-monthly cutting. Reference to the rainfall in Table III indicates that the first rains fell in late September, while Table C in the appendix shows not enough growth for a sample of these two grasses for the May to July period, and that in September the growth was green and short with 85 and 60 gm. respectively, for samples. In other words, the two-monthly samples consisted of entirely new growth of high phosphorus content. In case of four-monthly cuttings in September, the values of phosphorus given in Table IV are even more striking in that high values were obtained for all the grasses with the exception only of *Cynodon dactylon*, the sample which happened to be described in Table C as " mixed with flower heads " instead of " green short ", which applies to all the other samples for that period. The analyses of the five-monthly cuttings in Table IV read in conjunction with the description of the samples given in Table C, brings out the same point, viz., that stage of growth, depending in the first instance on climatic conditions, of which rainfall appeared to be the deciding factor, determined the phosphorus content of the grasses studied. As the grasses matured, the phosphorus content dropped, and under similar climatic conditions the phosphorus content of one month's growth would be higher than that of two months, while the latter is slightly higher than a three months' growth, although, after three months' growth, with an average figure of about ·2 per cent. P_2O_5 (see also Table II) there appears to be a variation in the P_2O_5 content between ·2 and about ·06 depending on the amount of new growth present and, therefore, included in the sample for analysis.

Comparing Tables II and IV, one is forced to the conclusion that a rise in the phosphorus content of the grasses was definitely brought about by periodic cutting after three, four, five and six months' growth when compared with the undisturbed growth for

TABLE V.

CRUDE PROTEIN CONTENT OF GRASSES.

(Time of growth: 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	10·0	11·7	10·8	12·3	7·9	12·5	16·8	12·4	14·2	10·9	12·3
March	7·5	10·2	8·5	8·0	5·2	9·5	9·7	6·7	6·6	7·1	8·0
April	6·0	8·0	6·5	5·5	4·6	6·0	6·2	4·2	6·2	4·0	6·0
May	4·9	7·1	5·6	4·0	2·7	5·0	6·5	3·1	4·6	3·6	6·2
June	4·2	5·5	5·0	3·5	2·3	3·8	5·4	3·7	3·5	2·5	6·7
July	4·6	4·9	3·5	3·6	2·3	3·5	4·5	2·7	2·1	1·9	4·0
August	4·4	4·8	3·0	4·4	2·4	3·3	4·9	5·2	3·4	4·2	5·0
September	4·0	5·7	4·2	5·0	2·5	5·9	6·1	3·0	3·2	2·9	5·5
October	5·9	7·7	5·2	4·4	2·7	5·1	6·8	3·2	5·1	4·1	8·5
November	4·1	5·9	5·0	5·6	2·6	5·2	8·8	3·2	4·3	4·6	7·2
December	5·2	12·6	5·1	5·2	4·8	6·9	7·5	4·1	4·1	9·0	7·8
1933.											
January	5·0	11·5	4·9	6·5	4·7	5·3	9·4	7·0	4·1	5·7	8·4

the same periods given in Table I. For instance, a removal of the old grasses by cutting in June (Table IV five-monthly cutting) had greater effect on the composition of the next five months' growth in November than leaving the grass undisturbed and analysing it after 10 months' growth (cf. Table II, November). Obviously, the analyses will have to be carried out for several years to confirm the tentative conclusion mentioned above. Much more important is the observation that the grasses reached maturity and, therefore, a low value for phosphorus, very rapidly, and remained low in phosphorus for the rest of the twelve months' period in spite of new growth having taken place from time to time.

PROTEIN.

The protein content of the grasses studied is given as indicated in Table V.

Table V reveals that the values for protein were highest after one month growth, dropped quite rapidly as in the case of phosphorus, until mid-winter, when they ranged round about a quarter of the original figure, then rose during spring, apparently when new growth began. It seems that the protein content of the grasses responded remarkably well to new growth, several values in January being approximately double those of July. As in the case of phosphorus, the protein values after one month's growth were higher than any other given in Table V. The younger the plant the higher apparently is the protein content, or again stage of growth determines ultimately the protein content of the plant.

Table VI presents further data on the protein content of the grasses as indicated.

There is no doubt that with the climatic conditions existing in 1932 the protein content of the grasses studied remained definitely higher when cut at intervals as stated in Table VI than when allowed to grow according to the scheme of analysis set forth in Table V. It is realized, of course, that increase in the protein, phosphorus and, maybe, other constituents of the plant by periodic cutting, has not much practical value unless the yield of grass is not too adversely affected by the periodic cutting. The plots were too small to study yield as already stated, although the weights of the grass samples obtained at each cutting as given in Tables A, B and C in the appendix, give some indication of the quantity of grass obtained, when cutting, for instance, every two months on six occasions, compared with that obtained for one cutting at the end of 12 months. On the whole, one cutting after twelve months produces from one and a half to twice as much grass as the total of six two-monthly cuttings. Furthermore, although the yield is much greater, the proportion of unpalatable hard fibrous grass in the long period sample is also much greater than in that cut at shorter intervals. There is the possibility that a smaller yield will be fully compensated for by better quality in the short term growth. This point, about improvement in quality at the expense of yield, will be considered at a later stage in the work under consideration when enough data have accumulated over several years under different climatic conditions. For the present, it can only be said that the protein content of

TABLE VI.

CRUDE PROTEIN CONTENT OF GRASSES.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	10·0	11·7	10·8	12·3	7·9	12·5	16·8	12·4	10·9	14·2	12·3
March	12·9	13·0	12·8	14·2	10·0	15·6	16·1	15·4	13·4	11·7	14·6
April	13·2	11·7	14·0	14·1	12·2	16·7	16·2	15·4	13·2	—	14·8
May	—	—	13·7	—	—	—	—	—	14·8	—	—
August	—	—	12·4	—	—	—	—	—	—	12·7	—
September	—	—	12·6	16·0	—	—	—	—	13·3	9·5	19·2
October	11·2	10·5	11·5	13·9	11·5	16·1	20·1	—	15·0	10·3	21·6
November	13·6	11·1	10·9	13·1	10·1	16·2	18·8	13·0	13·9	11·5	22·3
December	11·8	21·5	18·4	13·6	16·4	15·8	18·3	15·8	15·3	11·9	18·9
1933 January	—	21·3	11·7	12·8	10·5	14·7	—	—	20·7	—	—
Two-monthly Growth.											
1932 March	7·5	10·2	8·1	8·0	5·2	9·5	9·7	6·7	7·1	6·6	8·0
May	11·1	7·9	11·3	10·0	11·2	13·7	15·8	11·5	10·7	10·0	12·7
July	10·7	—	12·8	—	10·5	—	—	—	10·8	—	—
September	—	—	12·8	18·2	11·8	13·0	—	—	14·8	11·6	17·2
November	9·0	12·1	7·4	8·3	7·7	12·3	16·8	10·7	11·5	7·2	13·6
1933 January	9·0	11·0	10·9	12·3	11·4	—	14·7	10·3	10·6	14·2	—
Three-monthly Growth.											
1932 April	6·0	8·0	6·5	5·5	4·6	6·0	6·2	4·2	4·0	6·2	6·0
July	10·8	5·6	12·9	13·6	9·2	15·7	16·1	13·8	10·0	6·7	16·9
October	10·7	12·5	10·0	7·7	9·4	12·1	16·8	9·3	13·0	9·7	20·9
1933 January	13·1	16·0	6·7	—	10·4	9·0	12·9	—	10·0	7·6	—
Four-monthly Growth.											
1932 May	4·9	7·1	5·6	4·0	2·7	5·0	6·5	3·1	3·6	4·6	6·2
September	12·0	—	11·0	12·9	15·1	22·0	18·3	—	16·4	10·5	27·0
1933 January	8·2	9·3	6·6	10·6	11·4	12·3	12·2	12·8	—	5·5	13·7
Five-monthly Growth.											
1932 June	4·2	5·5	5·0	3·5	2·3	3·8	5·4	3·7	2·5	3·5	6·7
November	10·0	10·2	10·9	12·1	8·3	11·2	14·8	11·1	10·8	10·4	16·3
Six-monthly Growth.											
1932 July	4·6	4·9	3·5	3·6	2·3	3·5	4·5	2·7	1·9	2·1	4·0
1933 January	7·4	8·7	5·3	7·0	7·3	12·3	12·1	7·8	9·4	8·9	12·9

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TABLE VII.
CRUDE FIBRE CONTENT OF GRASSES.

(Time of growth: 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhena hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	36·4	28·7	34·0	35·0	37·2	33·3	28·5	35·7	33·0	34·5	29·5
March	39·4	27·7	36·8	39·3	43·4	36·1	33·5	40·0	37·6	36·4	33·2
April	36·8	28·8	36·2	·38·3	41·8	34·2	33·4	40·9	36·0	34·3	33·4
May	38·6	28·1	33·0	37·9	41·6	35·5	33·1	41·0	32·8	37·2	28·4
June	39·8	28·4	33·4	36·7	40·5	35·5	33·8	40·1	32·6	34·9	30·8
July	38·3	29·2	36·3	35·4	41·0	34·5	34·8	42·7	36·9	34·6	32·9
August	37·6	31·1	35·7	37·4	41·5	34·1	34·0	41·6	31·9	34·8	30·8
September	39·8	28·6	35·7	32·7	41·7	35·9	32·3	41·3	34·2	34·7	34·1
October	34·5	28·5	31·1	36·4	41·8	34·3	31·3	39·8	35·9	35·7	32·3
November	37·2	26·7	32·1	33·1	42·1	32·6	29·6	40·1	36·3	34·4	29·2
December	34·7	28·6	33·9	36·4	39·1	36·5	32·2	38·0	32·7	33·5	28·0
1933.											
January	38·7	31·0	36·4	35·2	35·8	35·1	30·3	39·6	33·8	33·5	31·4

samples cut at monthly intervals (Table VI) is enough to provide for the requirements of a 2-gallon cow under ranching conditions, and that, judging from the eleven species of grasses studied, the protein content does not vary as much from species to species as from one stage of growth to another.

FIBRE.

Tables VII and VIII contain data on the fibre content of eleven species of grasses at various stages of growth.

Hyparrhenia hirta and *Rhynchelythrum roseum* are highest in fibre according to Table VII, although *Amphilophis insculpta* used for making hay sometimes, and *Cymbopogon plurinodes*, or turpentine grass, are not much lower, and are almost on a par with what are commonly called good grasses, like *Panicum maximum, Urochloa pullulans, Eragrostis superba, etc.* As a matter of fact, the fibre content of all the grasses given in Table VII are high. When cut at intervals of one month, as stated in Table VIII, the fibre content of the grasses decreases from about 35 per cent. to approximately 25 per cent., although according to Woodman's work (1926 *et seq.*) it is doubtful whether the digestibility improves accordingly. Increase in fibre lowers palatability, and is also undoubtedly associated with advance of stage of growth which in its turn, as will appear from the analyses, indicates a decrease in the " quality " of the grass. The association of fibre content with palatability, is very important, but cannot be considered in this investigation. Finally, a consideration of digestibility in relation to increase in fibre, i.e. advance of stage of growth, is of the utmost importance. Woodman (1932) states that during a season of favourable rainfall five-weekly cutting of herbage prevented greatly reduced digestibility as increased lignification was practically negligible. This may not apply to South African conditions where growth is more rapid under conditions of favourable rainfall and lignification may set in sooner than at Cambridge.

Further information will be obtained on the question of maturation of pasture and its effect on digestibility early next spring, when digestibility trials on pasture at different stages of growth will be undertaken.

CALCIUM.

The calcium content of the eleven species of grasses is given as indicated in Tables IX and X.

With the doubtful exception of *Cynodon dactylon*, there is apparently a slight but inconsistent drop in the calcium content of the grasses as they mature. This tendency for calcium to be lower on the whole for advanced stages of growth is more apparent if the figures for the calcium content of monthly growth in Table X are compared with the corresponding figures given in Table XI for the same grass from one month old up to twelve months.

TABLE VIII.
CRUDE FIBRE CONTENT OF GRASSES.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulanes.
Monthly Growth.											
1932 February	36·4	28·7	34·0	35·0	37·2	33·3	28·5	35·7	34·5	33·0	29·5
March	32·8	27·1	30·4	28·8	30·0	30·4	26·6	30·3	32·7	30·8	25·6
April	32·4	25·6	29·0	26·6	32·4	28·3	25·2	29·1	28·3	—	23·2
May	—	—	26·7	—	—	—	—	—	24·3	—	—
August	—	—	26·4	25·7	28·1	26·1	19·7	—	—	27·0	—
September	29·8	24·7	26·9	29·0	28·6	22·0	21·0	29·8	26·1	31·3	17·2
October	30·4	26·1	26·8	27·9	31·1	28·4	22·8	30·1	27·1	28·3	16·6
November	32·4	27·8	29·8	29·7	28·3	29·2	24·0	—	29·0	29·2	19·9
December		26·2	28·3	28·3					32·4	28·4	19·2
1933 January									30·5		
Two-monthly Growth.											
1932 March	39·4	27·7	36·8	39·3	43·4	36·1	33·5	40·0	36·4	37·6	33·2
May	30·5	27·7	26·0	27·5	29·1	28·6	22·2	29·1	25·3	27·4	24·1
July	29·5	—	25·3	—	25·5	—	—	—	23·8	—	—
September	—	24·4	27·2	26·0	27·4	29·6	21·8	32·8	25·6	27·8	19·3
November	32·0	26·8	30·2	32·6	31·8	36·0	26·6	32·8	29·3	31·6	24·1
1933 January	34·7	—	34·6	33·6	34·7	33·1	—	—	32·0	31·0	—
Three-monthly Growth.											
1932 April	26·8	28·8	36·2	38·3	41·8	34·2	33·4	40·9	34·3	36·0	33·4
July	29·2	28·5	25·2	—	27·0	27·0	24·2	—	24·5	27·7	—
October	30·2	25·0	30·6	30·6	30·1	27·6	21·2	26·4	25·7	32·6	17·4
1933 January	31·9	28·4	33·2	33·0	34·3	32·8	26·5	32·0	32·2	31·2	23·0
Four-monthly Growth.											
1932 May	38·6	28·1	33·0	37·9	41·6	35·5	33·1	41·0	37·2	32·8	28·4
September	30·4	27·0	26·7	26·6	25·6	28·6	20·7	—	24·2	28·3	16·5
1933 January	30·1	27·3	33·1	33·9	31·8	33·3	26·6	32·2	30·1	31·2	22·1
Five-monthly Growth.											
1932 June	39·8	28·4	33·4	36·7	40·5	35·5	33·8	40·1	34·9	32·6	30·8
November	29·8	25·8	30·3	34·3	28·3	27·5	27·3	28·0	27·3	31·8	16·1
Six-monthly Growth.											
1932 July	38·3	29·2	36·3	35·4	41·0	34·5	34·8	42·7	34·6	36·9	32·9
1933 January	32·5	26·6	34·8	31·2	31·3	30·7	25·3	40·3	30·0	33·5	22·6

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TABLE IX.

CaO Content of Grasses.

(Time of growth: 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	·51	·54	·35	·52	·32	·33	·81	·52	·51	·43	·74
March	·43	·57	·30	·41	·29	·26	·41	·30	·39	·36	·54
April	·47	·61	·39	·48	·35	·34	·51	·32	·52	·45	·48
May	·45	·63	·39	·46	·30	·48	·78	·28	·59	·45	·44
June	·51	·60	·43	·59	·33	·38	·66	·36	·58	·61	·51
July	·55	·77	·40	·47	·34	·33	·58	·38	·55	·60	·65
August	·50	·77	·33	·42	·44	·29	·81	·38	·72	·61	·71
September	·45	·71	·34	·51	·36	·39	1·12	·44	·52	·65	·74
October	—	·64	·36	·36	·32	·47	·67	·42	·50	·57	·90
November	·44	·60	·40	·44	·29	·37	1·03	·43	·44	·65	·88
December	·50	·65	·32	·43	·38	·45	·81	·51	·44	·61	·89
1933.											
January	·53	·72	·30	·39	·28	·44	1·05	·50	·48	·67	·79

TABLE X.
CaO Content of Grasses.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhunchelythrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	·51	·54	·35	·52	·32	·33	·81	·52	·43	·51	·74
March	·53	·51	·34	·50	·39	·28	·64	·45	·40	·46	·65
April	·60	·58	·45	·62	·50	·43	·77	·58	·50	—	·88
May	—	—	·42	—	—	—	—	—	·55	—	—
August	—	—	·44	·80	—	—	—	—	—	·66	—
September	—	·63	·55	·60	·60	·56	1·23	—	·68	·53	1·12
October	·60	·67	·47	·68	·48	·52	1·26	—	·65	·57	·98
November	·63	·65	·46	·69	·53	·52	1·22	·62	·57	·68	·96
December	·61	·56	·42	·72	·54	·49	1·00	·72	·56	·70	·81
1933 January	—	—	·39	—	—	—	—	—	·61	—	—
Two-monthly Growth.											
1932 March	·43	·57	·30	·41	·29	·26	·41	·30	·36	·39	·54
May	·70	·50	·53	·93	·78	·54	1·17	·80	·79	·73	·93
July	·86	—	·62	—	·95	—	—	—	·87	—	—
September	—	—	·64	·78	·74	·57	1·21	—	·69	·72	1·15
November	·57	·71	·47	·62	·59	·55	·89	·63	·71	·59	·83
1933 January	·63	·58	·36	·61	·47	·49	—	·57	·67	·67	—
Three-monthly Growth.											
1932 April	·47	·61	·39	·48	·35	·34	·51	·32	·45	·52	·48
July	·91	·55	·66	—	1·01	·71	1·32	—	1·05	·88	—
October	·76	·69	·49	·60	·57	·64	1·05	·70	·81	·48	1·08
1933 January	·63	·59	·38	·53	·43	·52	·90	·55	·66	·61	·76
Four-monthly Growth.											
1932 May	·45	·63	·39	·46	·30	·49	·78	·38	·45	·59	·44
September	·79	·61	·69	·87	·84	·63	1·19	—	·69	·59	·88
January	·60	·62	·41	·51	·46	·49	1·01	·52	·69	·56	·74
Five-monthly Growth.											
1932 June	·51	·60	·43	·59	·33	·38	·66	·36	·61	·58	·51
November	·70	·71	·54	·63	·71	·58	1·25	·63	·80	·53	·99
Six-monthly Growth.											
1932 July	·55	·77	·40	·47	·34	·33	·58	·38	·60	·55	·65
1933 January	·55	·58	·39	·51	·45	·43	1·02	·49	·69	·48	·77

TABLE XI.

Na$_2$O Content of Grasses.

(Time of growth : 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	·04	—	·035	·075	·02	066	·38	·057	—	·06	1 01
March	·03	·014	·02	·02	013	·04	·90	·027	·015	·012	47
April	·02	·029	·015	·02	014	·02	·73	·02	·02	·019	42
May	·01	·02	·014	·03	·023	019	·40	016	013	·016	·42
June	·046	·026	·012	·02	·02	·016	·44	·017	009	·019	·23
July	·04	·024	·031	·02	·028	·04	·27	·022	·016	·025	058
August	·04	·029	·018	·03	·026	·06	·23	·026	·022	·025	·155
September	·04	·036	·024	·03	·02	·04	117	·021	·016	·022	10
October	—	·042	·029	·03	·02	·043	·29	·012	·01	·044	·245
November	025	·041	·027	·026	017	035	·33	·01	·01	·032	06
December	027	·037	·028	·025	·02	·04	·35	·018	·016	·036	·047
1933.											
January	·036	·032	·031	·023	·02	·047	·356	·02	·022	·046	056

TABLE XII.
Na$_2$O Content of Grasses.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	·04	—	·035	·075	·02	·066	·38	·057	·06	—	1·01
March	·027	·016	·035	·047	·019	·067	·47	·07	·03	·03	·70
April	·034	·029	·038	·026	·025	·05	·51	·048	·034	—	·70
May	—	—	·025	—	—	—	—	—	·15	—	—
August	—	—	·076	—	—	—	—	—	—	—	—
September	—	—	·036	·049	—	·064	·84	—	·039	·043	1·18
October	·079	·036	·023	·044	·027	·055	·73	·035	·045	·038	·54
November	·086	·055	·04	·04	·033	·096	·55	·047	·04	·036	·50
December	·09	·039	·048	·027	·03	·057	·87	—	·049	·03	·60
1933 January	—	·037	·056	·036	·029	—	—	—	·052	·04	—
Two-monthly Growth.											
1932 March	·03	·014	·02	·02	·013	·04	·90	·027	·012	·015	·47
May	·02	·02	·016	·02	·02	·03	·47	·127	·03	·027	·49
July	·04	—	·037	—	·039	—	—	—	·056	—	—
September	—	—	·028	·05	·025	·041	·66	·032	·034	·038	·50
November	·034	·039	·039	·035	·03	·041	·63	·039	·043	·019	·26
1933 January	·052	·045	·044	·035	·022	·06	—	—	·048	·027	—
Three-monthly Growth.											
1932 April	·02	·029	·015	·02	·014	·02	·73	·02	·019	·02	·42
July	·04	·027	·027	—	·035	·048	·31	—	·035	·024	—
October	·047	·042	·026	·049	·031	·059	·60	·039	·040	·03	·51
1933 January	·044	·041	·029	·036	·025	·051	·50	·043	·045	·026	·17
Four-monthly Growth.											
1932 May	·01	·02	·014	·03	·023	·019	·40	·016	·016	·013	·42
September	·047	·018	·026	·04	·029	·032	·315	—	·059	·044	·516
1933 January	·053	·032	·042	·029	·023	·054	·37	·029	·048	·015	·115
Five-monthly Growth.											
1932 June	·046	·026	·012	·02	·02	·016	·44	·017	·019	·009	·23
November	·04	·036	·033	·024	·025	·025	·45	·041	·039	·022	·32
Six-monthly Growth.											
1932 July	·04	·024	·031	·02	·028	·04	·27	·022	·025	·016	·058
1933 January	·036	·029	·038	·025	·02	·058	·33	·033	·055	·013	·10

There is a remarkable variation in the calcium content of different species of grasses at different stages of growth. *Cymbopogon-plurinoides* or turpentine grass, *Hyparrhenia hirta* or thatch grass, and *Rhynchelythrum roseum*, all show poor values while *Urochloa pullulans* and *Panicum maximum*, which make good grazing, are high.

If these eleven species of grasses are at all indicative of the calcium content of natural grazing, it is extremely unlikely that stock will suffer from a calcium deficiency under ranching conditions. A cow producing 2 gallons of milk would consume under grazing conditions about four times as much calcium as she secretes in her milk. Even mature grasses according to Table IX are fairly high in calcium if hard fibrous grasses like *Cymbopogon plurinoides* and *Hyparrhenia hirta*, which in any case animals will not touch at that stage of growth, are omitted.

Sodium.

The sodium contents of the grasses studied are given in Tables XI and XII.

A very significant feature of the sodium content of the grasses is the great variation in sodium for different species at the same stage of growth. *Urochloa pullulans* and *Panicum maximum* are outstandingly high in sodium, while the other species vary from about ·01 to ·04 per cent., which does not appear to vary for different stages of growth. *Urochloa* shows a remarkable drop in Table XI, which is not so pronounced for *Panicum maximum*.

The value for sodium appear to be remarkably low on the whole, and Table XI suggests that the intake of sodium by cattle on grazing certainly justifies serious consideration of a possible deficiency of this constituent. Basing the sodium requirements of a cow again on a two-gallon production standard approximately 10 gm. of Na_2O are secreted in her daily milk, while 25 lb. of grazing on the dry basis contain approximately 11 gm. of Na_2O, which have to suffice for milk production as well as for the maintenance of the cow. ·025 gm. Na_2O for the composition of pasture eaten is obviously an arbitrary figure, but is sufficiently correct to bring out the possibility of too little sodium being present in pasture for milk production without supplementary feeding. Later results, especially when mixed pasture is being considered, will throw more light on this problem of a possible sodium deficiency.

Potassium and Chlorine.

Potassium and chlorine may be taken together for the purpose of discussion as they show great similarity in at least two respects in the Tables XIII, XIV, XV and XVI, which give the results as indicated : —

TABLE XIII.

K$_2$O Content of Grasses.

(Time of growth : 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	2·57	1·87	2·66	2·8	2·13	5·4	3 50	3·40	2·28	3·88	3·04
March	1·91	1·34	1·99	1·78	1·61	3·6	1·89	2·07	1·55	2·34	2·74
April	1·30	1·00	1·14	1·23	1·28	2·6	1·60	1·55	1·20	1·62	2·45
May	1·19	·94	1·05	·99	1·05	1·92	1·70	1·28	1 05	1·84	2·30
June	1·20	·70	1·01	·84	·98	1·62	1 84	1·27	1·08	2·05	2 30
July	1·17	·65	·81	·91	·77	1 56	1·75	1·20	1·04	1·36	2·15
August	1·24	·69	·84	·61	·74	1·51	1·59	1·35	1·04	1·52	2·22
September	·94	·36	·64	·64	·45	1·20	1·04	·73	·68	·78	1·47
October	—	·76	·65	·78	·50	1·10	1·18	·47	·97	·86	·93
November	·68	·64	·83	·92	·55	1·39	·91	·42	·65	·95	1·15
December	·69	·99	·76	·82	·59	1·45	1·33	·33	·70	·97	1·20
1933.											
January	1·15	·99	·70	·88	·79	1·35	1·35	·38	·69	1·12	2·14

TABLE XIV.
K₂O Content of Grasses.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	2·57	1·87	2·66	2·8	2·13	5·4	3·50	3·40	3·88	2·28	3·04
March	2·20	1·65	2·42	2·51	2·25	4·96	2·81	3·93	3·52	1·82	3·47
April	2·01	1·32	2·28	1·93	1·94	4·85	2·23	2·57	2·67	—	3·24
May	—	—	2·83	—	—	—	—	—	2·55	—	—
August	—	—	2·10	—	—	—	—	—	—	—	—
September	—	1·64	1·69	2·24	1·62	4·95	2·05	—	2·98	1·73	3·05
October	2·09	1·44	2·16	2·20	1·75	5·00	2·75	2·37	3·47	1·83	4·44
November	2·30	1·31	2·41	1·92	1·64	4·75	2·67	2·46	3·48	1·87	3·63
December	2·42	1·53	2·22	1·54	1·86	4·00	2·91	—	2·97	1·44	4·00
1933 January			2·59	1·96					3·07	1·39	
Two-monthly Growth.											
1932 March	1·91	1·34	1·99	1·78	1·61	3 6	1·89	2·07	2·34	1·55	2·74
May	1·92	1·04	2·01	1·45	1·83	4·31	2 00	1·52	2·30	1·37	2·07
July	1·01	—	2·41	—	1·74	—	—	—	2·07	—	—
September	2·02	1·33	1·85	1·98	1·80	3·65	2·70	1·80	3·10	1·53	3·81
November	1·83	1·42	1·78	1·56	1·78	4·90	2·56	1·87	3·13	1·50	3 22
1933 January			1·81	·57	1·65	3·10			2·77	1·38	
Three-monthly Growth.											
1932 April	1·30	1·00	1·14	1·23	1·28	2 6	1·60	1·55	1·62	1·20	2·45
July	1·58	·78	1·69	—	1·40	2·60	2·00	—	1·59	1·27	—
October	2·07	1·29	1·67	2·03	2·12	3·87	2·59	2·33	4·50	1·70	3·90
1933 January	1·68	1·50	1·50	1·41	1 61	2·87	2·52	1·71	2·89	1·16	3·56
Four-monthly Growth.											
1932 May	1·19	·94	1·05	·99	1·05	1·92	1·70	1·28	1·84	1·05	2·30
September	1·40	·77	1·57	1·78	1·43	4·52	2·30	—	4·10	1·51	7·8
1933 January	1·82	1·29	1·44	1·21	1·76	2·53	2·31	1·41	2·62	·94	3·55
Five-monthly Growth.											
1932 June	1·20	·70	1·01	·84	·98	1·62	1·84	1·27	2·05	1·08	2 30
November	1·95	1·27	1·62	1·36	1·72	3·76	2·28	1·67	3·43	1·38	1·89
Six-monthly Growth.											
1932 July	1·17	·65	·81	·91	·77	1·56	1·75	1·20	1·36	1·04	2·15
1933 January	1·63	1·24	4·92	1·14	1·38	2·52	2·51	1·28	2·47	1·03	3·66

TABLE XV.
Cl Content of Grasses.

(Time of growth: 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	·91	·27	·53	·49	·47	·64	·82	·42	·54	·55	1·21
March	·73	·22	·38	·34	·39	·55	·91	·41	·44	·62	1·10
April	·39	·12	·27	·16	·26	·48	·85	·24	·33	·42	1·03
May	·36	·14	·27	·16	·27	·45	·79	·20	·27	62	·89
June	·36	·14	—	·13	·26	·38	·30	·23	·31	·59	·80
July	·39	·15	35	·11	·19	·29	·81	·17	·34	·51	·71
August	·33	·13	·26	·07	·23	·31	·63	·17	·34	·56	·68
September	·18	·09	·22	·10	10	·26	40	·085	·15		·60
October	—	·17	·22	·17	10	·35	·50		27		·2
November	·16	136	·25	24	13	·31	·43	·06	·18	39	?
December	·21	175	22	·19	·19	·27	·65	·04	·18	·36	
1933.											
January	·41	·23	23	·24	·08	·26	·52	·046	·25	·38	2 5

TABLE XVI.
Cl CONTENT OF GRASSES.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum cilare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	·91	·27	·53	·49	·47	·64	·82	·42	·55	—	·21
March	·88	·30	·49	·40	·56	·56	·74	·44	·59	—	·00
April	·66	·21	·34	·28	·40	·71	·78	·40	·55	—	·84
May	—	—	·44	—	—	—	—	—	·65	—	—
August	—	—	·39	·49	—	—	—	—	—	—	—
September	—	·33	·41	·44	·48	—	·76	·48	·58	·41	1·16
October	·76	·30	·46	·45	·47	·82	·88	·46	·74	·47	1·17
November	·76	·23	·48	·34	·45	·82	·75	—	·71	·46	·89
December	·68	·30	·39	·42	·56	·79	·85	—	·59	·39	·94
1933 January	—	—	·53	—	—	·89	—	—	·71	·48	—
Two-monthly Growth.											
1932 March	·73	·22	·38	·34	·39	·55	·91	·41	·62	·44	1·10
May	·75	·18	·42	·36	·61	·98	·84	·43	·76	·38	·96
July	·94	—	·48	—	·79	—	—	—	·65	—	—
September	—	—	·47	·50	·57	·73	1·01	·38	·74	·39	1·11
November	·84	·30	·43	·33	·52	·97	—	—	·80	·43	—
1933 January	·83	·31	·48	·34	·50	·97	·92	·39	·83	·50	·95
Three-monthly Growth.											
1932 April	·39	·12	·27	·16	·26	·48	·85	·24	·42	·33	1·03
July	·89	·14	·66	—	·78	·96	1·57	—	·77	·54	—
October	·82	·31	·55	·46	·68	1·19	1·05	·59	1·15	·53	1·47
1933 January	·61	·32	·36	·32	·42	·90	·91	·40	·84	·40	1·04
Four-monthly Growth.											
1932 May	·36	·14	·27	·16	·27	·45	·79	·20	·62	·27	·89
September	·61	1·16	·54	·46	·66	·93	·87	—	·93	·44	3·05
1933 January	·67	·30	·44	·29	·45	·76	·85	·28	·71	·29	1·10
Five-monthly Growth.											
1932 June	·36	·13	—	·13	·26	·38	·30	·23	·59	·31	·80
November	·76	·29	·45	·35	·54	·93	·92	·38	1·03	·38	1·27
Six-monthly Growth.											
1932 July	·39	·15	·35	·11	·19	·29	·81	·17	·51	·34	·71
1933 January	·65	·32	·35	·29	·37	·68	·90	·28	·68	·28	1·07

Both potassium and chlorine show a remarkable drop as the grasses mature.

It would seem that the drop should be greatest during the winter months, especially after the first heavy frosts have killed the grasses. That is, however, not the case, and it is especially noticeable in the case of potassium, where the minimum is reached from September to November (see Table XIII), in spite of new growth of high potassium content that took place after the first rains in September and October. It appears that the new growth was insufficient in bulk to increase the percentage potassium in the whole sample, which consisted of growth from January to May plus that from September to the time of sampling. Also, although new growth gradually increased from September onwards, the potassium content does not show marked response. The new growth in most cases, as is evident from Table XIV, remains fairly high in potassium up to six months old, so that the sample for analyses of *Amphilophis insculpta* (Table XIII) for November for instance, would be made up of new growth fairly high in potassium $\pm 1 \cdot 95$ (Table XIV), plus old growth, which took place before July and which at that stage showed $1 \cdot 17$ per cent. K_2O. Obviously, the figure actually obtained for the November sample, viz., $\cdot 68$, is lower and suggests that the old growth of the sample must have contained less K_2O than it did in July. In other words, the process of losing potassium, whether this be returned to the soil as Henrici (1930) reported for phosphorus, or whether this be due to the old grass tuft being exposed and losing, due to climate, more leaves than stalks, comparatively poorer in K_2O or whether rain leaches out the potassium, this process of decreasing the percentage K_2O in the old grass seems to continue into late spring or early summer. This point will be investigated further during the present year when new growth begins. From the data available it appears that potassium, chlorine and phosphorus, are more involved in this loss than the other constituents determined.

Tables XIII to XVI suggest that a chlorine or potassium deficiency at any stage of growth is probably entirely unwarranted in animals on pasture only when considering the production of anything up to two gallons of milk per day under ranching conditions.

The potassium content of different species of grasses varies remarkably at the same stage of growth. Less variation is shown in the case of chlorine.

MAGNESIUM.

Magnesium is present in the grasses studied in high concentrations as revealed by the following tables:—

TABLE XVII.
MgO Content of Grasses.
(Time of growth : 1 month, 2 months, 3 months, etc., up to 12 months.)

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Themeda triandra.	Setaria Gerrardii.	Urochloa pullulans.
1932.											
February	·38	·24	·26	·38	·23	·47	·73	·56	·23	·42	·66
March	·31	·20	21	·31	·21	·49	·69	·37	·18	·36	·45
April	·35	·33	·27	·29	·25	·61	72	·43	·29	·42	·51
May	·31	30	·25	·26	·22	·72	·72	·35	·28	·40	·48
June	·31	·31	·30	·33	·22	·65	·76	·40	·27	·53	·54
July	·35	·39	·23	·27	·23	·56	·78	·48	·28	·46	·48
August	·35	·40	·16	·26	·32	·56	·88	·41	·30	·47	·54
September	·29	·36	·23	·27	·22	·51	·94	·31	·28	·35	·47
October	—	·42	·22	·21	·27	·55	·63	·33	·22	·39	·62
November	·29	·39	·21	·22	·19	41	·85	·30	·27	·33	·52
December	·35	·43	·24	·24	·23	·50	·73	·31	·21	·39	·57
1933.											
January	·33	·45	·25	·25	·16	41	·82	·39	·27	·45	·64

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TABLE XVIII.
MgO Content of Grasses.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelythrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
Monthly Growth.											
1932 February	·38	·24	·26	·38	·23	·47	·73	·56	·42	·23	·66
March	·41	·21	·24	·37	·28	·51	·78	·54	·40	·24	·52
April	·55	·30	·34	·56	·46	·66	·98	·85	·55	—	·78
May	—	—	·34	—	—	—	—	—	·64	—	—
August	—	—	·32	—	—	—	—	—	—	—	—
September	·45	·50	·41	·69	·49	·89	1·38	—	·49	·27	1·33
October	·47	·49	·33	·51	·40	·78	1·34	·82	·59	·29	1·17
November	·48	·40	·33	·53	·45	·84	1·29	·91	·51	·27	1·16
December	—	·36	·32	·57	·43	·91	1·10	—	·55	·30	1·00
1933 January	—	—	·35	·61	—	—	—	—	·65	·36	—
Two-monthly Growth.											
1932 March	·31	·20	·21	·31	·21	·49	·69	·37	·36	·18	·45
May	·54	·31	·33	·65	·55	·69	1·08	·85	·74	·34	·81
July	·59	—	·36	—	·61	—	—	—	·58	—	—
September	—	—	·36	·68	·52	·64	—	·80	·54	·30	—
November	·39	·47	·32	·50	·42	·77	1·34	·64	·67	·27	1·19
1933 January	·45	·38	·29	·49	·33	·80	1·03	—	·65	·35	·74
Three-monthly Growth.											
1932 April	·35	·33	·27	·29	·25	·61	·72	·43	·42	·29	·51
July	·59	·35	·44	—	·64	·68	1·24	—	·74	·46	—
October	·55	·46	·33	·35	·42	·90	1·25	·79	·69	·28	1·10
1933 January	·44	·42	·29	·34	·32	·73	·96	·60	·63	·30	·75
Four-monthly Growth.											
1932 May	·31	·30	·25	·26	·22	·72	·72	·35	·40	·28	·48
September	·49	·36	·40	·49	·59	·61	·93	—	·48	·27	·68
1933 January	·41	·39	·31	·31	·35	·69	·88	·51	·60	·31	·63
Five-monthly Growth.											
1932 June	·31	·31	·30	·33	·22	·65	·76	·40	·53	·27	·54
November	·45	·44	·33	·37	·53	·78	1·09	·64	·65	·27	·90
Six-monthly Growth.											
1932 July	·35	·39	·23	·27	·23	·56	·78	·48	·46	·28	·48
1933 January	·39	·39	·25	·33	·36	·60	·93	·48	·60	·26	·70

There is quite a marked variation in the magnesium content of the different species of grasses at the same stage of growth, as a glance at the tables given above reveals. Apparently the magnesium content of any particular species is not appreciably affected by the stage of growth, there being no noticeable drop in the values given in Table XVII from February, 1932, to January, 1933. It would seem, however, from Table XVIII that regular cutting does tend to keep the magnesium content of grasses at a higher level. This is especially so in the case of *Panicum maximum* and *Urochloa pullulans*, where the tendency for the values of the samples cut at monthly intervals is to lie round about one per cent. instead of between the lower limits given for these two grass species in Table XVII.

Further analyses and more data therefore are bound to shed further light on this point when more figures are available for consideration.

DISTRIBUTION OF THE CONSTITUENTS DETERMINED IN THE GRASSES.

Each of the samples of monthly, two-monthly, three-monthly, up to twelve-monthly stages of growth of four of the species of grasses, was divided into leaves, stalks, and inflorescence respectively, and these fractions separately analysed in order to determine the distribution of the constituents in the grasses. The four species of grasses selected were *Amphilophis insculpta*, *Eragrostis superba*, *Hyparrhenia hirta* and *Pennisetum ciliare* and the data are presented in Tables XIX to XXII hereunder:—

Two of the samples were unfortunately not divided up in October as was also not the sample of *Eragrostic superba* taken in March.

The proportion of stalks appears to be greater during the winter months, or in the case of two less leafy grasses, *Hyparrhenia hirta* and *Amphilophis insculpta* in spring after stalks of the new season's growth have been formed. In any case, an important omission was made after the winter of 1932 by not sub-dividing leaves and stalks and inflorescence each again into new growth and old growth respectively. This matter has been rectified in the samples for the present year. Such a sub-division may throw light on the cause of the continued low content, especially of phosphorus, potassium and chlorine, even after new growth had started. The values (Tables II, XII and XV) remain lower in spite of new growth, high in these minerals, than their respective values for the winter months. It is important, therefore, to distinguish in the analyses of the samples between old growth and new growth. A continued drop in the potassium content, for instance, of the old growth may be masked partly or wholly by the high potassium content of the new growth unless old growth is separated from new growth and separately analysed. Furthermore, the present system of sub-dividing the samples has not brought to light the reason for the continued drop in certain constituents, which vary appreciably with stage of growth even after the mature stage in winter had been reached. It would appear that the time of poorest grazing as far as phosphorus was concerned was not limited to the winter months, but extended in the case of some grasses into early summer well after new growth had taken place.

It is evident from Tables XIX to XXII that except in the case of *Hyparrhenia hirta* on many occasions not enough inflorescence was present to provide a sample for analysis. The constituents determined were present in greater concentration in the leaves than in the stalks and the difference between the leaf-content and the stalk-content of anyone constituent becomes more noticeable as the plant matures, or when old grass is present, e.g. after March, 1932. In the case of some constituents the difference between the leaf-content and stalk-content of a particular constituent is quite marked, e.g. phosphorus and calcium, and is less obvious in other cases, e.g. sodium.

GROWTH DURING WINTER, 1932.

A glance at any of the tables giving the analyses of monthly cuttings of the same part of a plot reveals that in none of the species did enough growth for analyses occur during the severest winter months. Some species, however, continued growing later into the winter and others started earlier in spring than did the rest.

Table B in the appendix presents the data in regard to growth during winter and response to regular monthly cutting of the eleven species of grasses in 1932.

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From a consideration of Table B there appears to be considerable variation in the response of the grasses to regular monthly cutting and to growth during the winter months. *Cymbopogon plurinodis* and *Setaria gerrardii* were undoubtedly the hardiest species, while *Rhynchelythrum roseum*, a coarse fibrous grass, practically died out as a result of the severe treatment.

GENERAL SUMMARY AND CONCLUSIONS.

The results of plot experiments are reported in this publication. Eleven species of grasses, grown on separate plots, were exposed to the same climatic conditions and analysed at monthly intervals according to the following plan: A portion of each plot was cut at monthly intervals so that the analyses for monthly cuts all the year round for each grass could be made. Another portion of the plot was cut at two-monthly intervals for the full period, each subsequently analysed, a third portion was cut at three-monthly intervals, a fourth at four-monthly intervals and so on, up to twelve months, when a sample of twelve-months' growth was taken off each plot and analysed. The results are presented in tabulated form and discussed for the period February, 1932, to January, 1933.

The samples were analysed for crude protein, fibre, phosphorus, calcium, sodium, potassium, magnesium and chlorine, and the results of each constituent in all the species of grasses, presented separately and discussed. 1932 was a dry year and the results obtained cannot be applied except in broad outline to other years. However, the work is continuing and will be presented from time to time.

Probably all the constituents determined in the grasses are affected by the stage of growth to a greater or smaller extent. Phosphorus, protein, chlorine, sodium and potassium diminished rapidly as the stage of growth of the grasses advanced from one month to maturity. This decrease was less noticeable or even doubtful in the case of magnesium, calcium and even fibre. Most of the constituents that show an appreciable drop in the grasses during the winter to no rise directly new growth begins in the samples composed of old pre-winter growth plus new growth, e.g. a sample of nine-monthly growth of any of the species. This point was rather baffling and is receiving special attention during the present season.

The composition of the soil was the same in all the plots so that the effect of soil fertility and composition on the composition of the grasses is not being considered.

The different species of grasses showed very remarkable variations in the content of constituent determined at the same stage of growth. Apparently, however, if a grass is high in any one constituent, it is high in the others, which make for good quality. For instance, *Panicum maximum* and *Urochloa pullulans* registered high values throughout, except for fibre and are undoubtedly the two best grasses considered, while *Hyparrhenia hirta* and *Rhynchelythrum roseum* have verified their reputations of being hard coarse grasses of low feeding value if eaten by stock. The above indicates the importance of determining both botanical composition and chemical composition when evaluating pasture.

Digestibility trials were not carried out on the grasses as the plots were too small for this type of work. Digestibility work on a number of grass species will, however, be carried out as soon as the growth of the new season begins.

The deplorably low phosphorus content of the grasses as the period of growth advances beyond a month as is usually the case in natural pasture, is emphasized. Under such conditions a phosphorus deficiency seems to be a foredrawn conclusion, as is borne out by the first report already published (1932) on a mineral survey of the Union. The analyses may suggest lower values than actually exist in the pasture as eaten, for in taking samples for analysis, no notice is taken of selective grazing by the animal.

The values for protein and sodium are low on the whole and suggest the possibility of both being present in inadequate amounts for production at certain times of the year. The other constituents appear to be present in abundance and the analyses of the grasses in question do not suggest a shortage of these constituents in natural pasture.

A number of additional plots have been planted with other species of grasses which are also being studied and analysed during the present year. A further publication will be made in due course.

REFERENCES.

DU TOIT, P. J., MALAN, A. I., LOUW, J. G., HOLZAPFEL, C. R., AND ROETS, G. C. S. (1932). A Study of the Mineral Content and Feeding Value of Natural Pastures in the Union of South Africa. *18th Rept. Dir. of Vety. Serv. and Anim. Indust.*, pp. 525-577.

HENRICI, M. (1930). The Phosphorus Content of Grasses in the Eastern Transvaal. *16th Rept. Dir. of Vety. Serv. and Anim. Indust.*, pp. 435-463.

HOLZAPFEL, C. R. (1934). Modifications of the Methods Used at Onderstepoort for the Determination of (a) Magnesium and Calcium, (b) Potassium, in Grass Extracts. *Onderstepoort Journ.*, Vol. 2, No. 1.

LOUW, J. G. (1933). The Colorimetric Determination of Sodium in Vegetation. *Onderstepoort Journ.*, Vol. 1, No. 2.

LOUW, J. G. (1933). Unpublished Data.

MALAN, A. I., AND VAN DER LINGEN, G. W. B. (1931). The Microdetermination of some Inorganic Elements in Blood and Vegetation. *17th Rept. Dir. Vety. Serv. and Anim. Indust.*, pp. 443-452.

WOODMAN, H. E., *et al.* (1926-1932). The Nutritive Value of Pasture. *J. Agric. Sci.*, 16-22.

APPENDIX.

(Numbers in Tables A, B, and C indicate yield of air-dry grass in grams.)

TABLE A.

SUCCESSIVE MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932.											
February	Green, with flower heads 497	Green, with flower heads 330	Green, with flower heads 215	Green, with flower heads 125	Green, with flower heads 307	Green, with flower heads 390	Green, with flower heads 285	Green, with flower heads 375	Green, with flower heads 355	Green, with flower heads 185	Green, with flower heads 353
March	Green, seeds falling out 760	Green, with flower heads 420	Green, seeds falling out 430	Green, with flower heads 950	Green, with flower heads 1482	Green, with flower heads 695	Green, seeds falling out 620	Green, seeds falling out 1250	Green, seeds falling out 700	Green, with flower heads 670	Green, seeds falling out 1300
April	Mixed, mainly green, seeds falling out 1110	Mixed, mainly green, seeds falling out 303	Mixed, mainly green, seeds falling out 570	Mixed, mainly green, seeds falling out 470	Mixed, mainly green, seeds falling out 1540	Mixed, mainly green, seeds falling out 874	Mixed, mainly green, seeds falling out 950	Mixed, mainly green, seeds falling out 1400	Mixed, mainly green, seeds falling out 750	Mixed, mainly green, seeds falling out 450	Mixed, mainly green, seeds falling out 1400
May	Mixed, mainly brown, seeds fallen out 1150	Mixed, seeds falling out 220	Mixed, mainly brown, seeds falling out 600	Mixed, seeds falling out 927	Mixed, seeds falling out 1572	Mixed, seeds falling out 780	Mixed, seeds fallen out 600	Mixed, seeds falling out 850	Mixed, mainly brown, seeds fallen out 550	Mixed, seeds falling out 550	Brown, seeds fallen out 1500
June	Brown, seeds fallen out 978	Mixed, mainly brown, seeds falling out 390	Mixed, mainly brown, seeds falling out 720	Mixed, mainly brown, seeds fallen out 730	Mixed, mainly brown, seeds falling out 1496	Mixed, mainly brown, seeds falling out 785	Mixed, mainly brown, seeds fallen out 750	Brown, seeds fallen out 1150	Brown, seeds fallen out 550	Mixed, mainly brown, seeds falling out 475	Brown, seeds fallen out 980
July	Brown, seeds fallen out 612	Mixed, mainly brown, seeds falling out 545	Brown, seeds falling out 755	Mixed, mainly brown, seeds fallen out 621	Brown, seeds fallen out 1475	Brown, seeds fallen out 623	Brown, seeds fallen out 705	Brown, seeds fallen out 980	Brown, seeds fallen out 575	Brown, seeds fallen out 425	Brown, seeds fallen out 935
August	Brown, seeds fallen out 1010	Brown, seeds falling out 520	Brown, seeds falling out 495	Brown, seeds fallen out 650	Brown, seeds fallen out 1766	Brown, seeds fallen out 605	Brown, seeds fallen out 720	Brown, seeds fallen out 1230	Brown, seeds fallen out 485	Brown, seeds fallen out 835	Brown, seeds fallen out 1110
September	Mixed, mainly brown, seeds fallen out 493	Mixed, mainly brown, seeds fallen out 675	Mixed, mainly brown, seeds fallen out 677	Mixed, mainly brown, seeds fallen out 637	Mixed, mainly brown, seeds fallen out 1165	Mixed, mainly brown, seeds fallen out 848	Mixed, mainly brown, seeds fallen out 805	Mixed, mainly brown, seeds fallen out 830	Mixed, mainly brown, seeds fallen out 485	Mixed, mainly brown, seeds fallen out 835	Mixed, mainly brown, seeds fallen out 1035
October	Mixed, mainly brown, new flower heads present 435	Mixed	Mixed, mainly brown, new flower heads fallen out 635	Mixed, mainly brown, new flower heads present 710	Mixed, mainly brown, new flower heads present 728	Mixed, new flower heads present 1220	Mixed, new flower heads present 810	Mixed, mainly brown, new flower heads present 680	Mixed, mainly brown, new flower heads present 370	Mixed, mainly brown, new flower heads present	Mixed, new flower heads present 1070
November	Mixed, with flower heads 1686	Mixed, mainly green, new flower heads 1120	Mixed, new flower heads present 12250	Mixed, with flower heads 1060	Mixed, with flower heads 2460	Mixed, with flower heads 1374	Mixed, mainly green, with flower heads 1220	Mixed, mainly brown, with flower heads 680	Mixed, with flower heads 370	Mixed, new flower heads present 995	Mixed, new flower heads present 1120
December	Mixed, mainly green, with flower heads 3816	Mixed, mainly green, with flower heads 1600	Mixed, with flower heads 4000	Mixed, with flower heads 4184	Mixed, with flower heads 4200	Mixed, with flower heads 3366	Mixed, with flower heads 2200	Mixed, with flower heads 2020	Mixed, with flower heads 1470	Mixed, with flower heads 1600	Mixed 2110
1933.											
January	Mixed, mainly green, seeds falling out 3060	Mixed, mainly green, with flower heads 2370	Mixed, mainly green, with flower heads 4145	Mixed, seeds falling out 4160	Mixed, seeds falling out 3299	Mixed, seeds falling out 4416	Mixed, mainly green, seeds falling out 1780	Mixed, mainly brown, seeds falling out 4230	Mixed, seeds falling out 1745	Mixed, with flower heads 1060	Mixed, mainly green, seeds falling out 4080

APPENDIX—(continued).

TABLE B.

ONE-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932.											
February....	Green, with flower heads 497	Green, with flower heads 330	Green, with flower heads 215	Green, with flower heads 125	Green, with flower heads 307	Green, with flower heads 390	Green, with flower heads 285	Green, with flower heads 375	Green, with flower heads 355	Green, with flower heads 185	Green, with flower heads 353
March........	Green, with flower heads 260	Green, with flower heads 170	Green, with flower heads 110	Green, with flower heads 200	Green, with flower heads 250	Green, with flower heads 230	Green, with flower heads 170	Green, with flower heads 120	Green, with flower heads 210	Green, with flower heads 70	Green, with flower heads 320
April........	Green, short. 130	Green, short, 95	Green, short, 60	Green, short, 100	Green, short, 120	Green, short, 120	Green, short, 70	Green, short, 90	Green, with flower heads 100	Green, short, little growth 100	Green, short, 100
May..........	Green, short, little growth	Green, short, little growth	Green, short. 50	Green, short, little growth	Green, short, little growth	Green, short, little growth	Green, short, little growth	Green, short, little growth	Green, short, 70	Green, short, little growth	Green, short, little growth
June.........	Practically no growth	Practically no growth	Green, short. little growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth
July.........	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth
August.......	Practically no growth	Practically no growth	Green, short, little growth 10	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Practically no growth	Green, no growth	Practically no growth
September....	Practically no growth	Green, short, little growth	Green, short, 35	Green, short, 60	Green, short, little growth	Green, short, little growth	Green, short, little growth	Practically no growth, some dying	Green, short, 35	Green, short, 30	Green, short, little growth
October......	Green, short, little growth	Green, short, 50	Green, short, 75	Green, with flower heads 150	Green, with flower heads 45	Green, short, 80	Green, short, 15	Green, short, some dying 45	Green, with flower heads 70	Green, with flower heads 115	Green, short, 55
November....	Green, short, 65	Green, short, 30	Green, with flower heads · 60	Green, with flower heads 130	Green, with flower heads 55	Green, short, 100	Green, short, 20	Green, with flower heads, some dying 35	Green, with flower heads 75	Green, short, 50	Green, short, 50
December.....	Green, with flower heads 75	Green, short, 45	Green, with flower heads 65	Green, with flower heads 135	Green, with flower heads 65	Green, with flower heads 160	Green, short, 30	Green, with flower heads, some dying	Green, with flower heads 115	Green, short, 55	Green, short, 75
1933.											
January......	Green, with flower heads 85	Green, short, 40	Green, with flower heads 45	Green, with flower heads 105	Green, with flower heads 55	Green, with flower heads 205	Green, with flower heads 35	Dead	Green, with flower heads 95	Green, with flower heads 45	Green, short,

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APPENDIX—(continued).

TABLE C.

TWO-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932. March......	Green, seeds falling out 760	Green, with flower heads 420	Green, seeds falling out 430	Green, with flower heads 950	Green, with flower heads 1482	Green, with flower heads 695	Green, seeds falling out 620	Green, seeds falling out 1250	Green, seeds falling out 700	Green, with flower heads 670	Green, seeds falling cut 1300
May.........	Mixed, mainly green, short 230	Green, short 115	Mixed, mainly green, short 150	Mixed, mainly green, short 90	Mixed, mainly green, short 170	Mixed, mainly green, short 205	Mixed, mainly green, short 50	Mixed, mainly green, short 100	Mixed, mainly green, short 130	Mixed, mainly green, short 100	Mixed, mainly green, short 75
July.........	Mixed, mainly brown 45	—	Green, short 30	Green, short 85	Green, short 30	Green, short 60	—	—	Green, short 23		—
September....	—	—	Green, short 95		Green, short 55				75	Green, short 65	
November....	Green, with flower heads 255	Green, with flower heads 60	Green, with flower heads 350	Green, with flower heads 475	Green, with flower heads 355	Green, with flower heads 335	Green, with flower heads 130	Green, with flower heads 170	Green, with flower heads 205	Green, with flower heads 295	Green, short 100
1933. January.....	Green, with flower heads 545	Green, with flower heads 215	Green, with flower heads 305	Green, with flower heads 585	Green, with flower heads 520	Green, with flower heads 930	Green, with flower heads 310	Green, with flower heads 225	Green, seeds falling out 255	Green, with flower heads 45	Green, with flower heads 285

THREE-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932. April........	Mixed, mainly green, seeds falling out 1110	Mixed, mainly green, seeds falling out 303	Mixed, mainly green, seeds falling out 570	Mixed, mainly green, seeds falling out 470	Mixed, mainly green, seeds falling out 1540	Mixed, mainly green, seeds falling out 874	Mixed, mainly green, seeds falling out 950	Mixed, mainly green, seeds falling out 1400	Mixed, mainly green, seeds falling out 750	Mixed, mainly green, seeds falling out 450	Mixed, mainly green, seeds falling out 1400
July.........	Mixed, mainly brown, short 70	Green, short 135	Green, short 85		Green, short 70	Mixed, mainly green, short 45	Mixed, mainly green, short 25		Green, short 25	Green, short 115	
October......	Green, with flower heads 125	Green, short 70	Green, with flower heads 305	Green, with flower heads 235	Green, with flower heads 270	Green, with flower heads 190	Green, short 115	Green, with flower heads 100	Green, with flower heads	Green, with flower heads 415	Green, short 130
1933. January.....	Green, seeds falling out 505	Green, with flower heads 205	Green, with flower heads 710	Green, seeds falling out 395	Green, with flower heads 855	Green, seeds falling out 805	Green, seeds falling out 985	Green, seeds falling out 330	Green, seeds falling out 330	Green, with flower heads 350	Green, with flower heads 465

MINERAL CONTENT AND FEEDING VALUE OF SOUTH AFRICAN PASTURES.

P. J. DU TOIT, A. I. MALAN, J. G. LOUW, C. R. HOLZAPFEL, AND G. C. S. ROETS.

APPENDIX—(continued).

TABLE C—(continued).

FOUR-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932. May.........	Mixed, mainly brown, seeds fallen out 1150	Mixed, mainly brown, seeds falling out 390	Mixed, mainly brown, seeds falling out 600	Mixed, seeds falling out 927	Mixed, seeds fallen out 1572	Mixed, seeds falling out 780	Mixed, seeds fallen out 600	Mixed, seeds falling out 850	Mixed, mainly brown, seeds fallen out 550	Mixed, seeds falling out 550	Brown, seeds fallen out 1500
September....	Green, short 50	Mixed, with flower heads 105	Green, short 125	Green, short 110	Green, short 45	Green, short 45	Green, short 20	Green, short	Green, short 30	Green, short	Green, short 25
1933. January......	Green, seeds falling out 670	Green, with flower heads 305	Green, with flower heads 630	Green, seeds falling out 720	Green, with flower heads 500	Green, seeds falling out 770	Green, seeds falling out 335	Green, seeds falling out 345	Green, seeds falling out 245	Green, with flower heads 505	Green, with flower heads 455

FIVE-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932. June.........	Brown, seeds fallen out 978	Mixed, mainly brown, seeds falling out 390	Mixed, mainly brown, seeds falling out 720	Mixed, mainly brown, seeds falling out 730	Mixed, mainly brown, seeds fallen out 1466	Mixed, mainly brown, seeds falling out 785	Mixed, mainly brown, seeds fallen out 750	Brown, seeds fallen out 1160	Brown, seeds fallen out 550	Mixed, mainly brown, seeds falling out 475	Brown, seeds fallen out 980
November....	Green, with flower heads 270	Green, short 130	Green, with flower heads 335	Green, with flower heads 485	Green, with flower heads 280	Green, with flower heads 300	Green, short 170	Green, with flower heads 175	Green, with flower heads 155	Green, with flower heads 430	Green, short 135

SIX-MONTHLY GROWTH.

	Amphilophis insculpta.	Cynodon dactylon.	Cymbopogon plurinodis.	Eragrostis superba.	Hyparrhenia hirta.	Pennisetum ciliare.	Panicum maximum.	Rhynchelytrum roseum.	Setaria Gerrardii.	Themeda triandra.	Urochloa pullulans.
1932. July.........	Brown, seeds fallen out 612	Mixed, mainly brown, seeds falling out 545	Brown, seeds falling out 755	Mixed, mainly brown, seeds falling out 621	Brown, seeds fallen out 1475	Brown, seeds fallen out 635	Brown, seeds fallen out 765	Brown, seeds fallen out 980	Brown, seeds fallen out 595	Brown, seeds fallen out 425	Brown, seeds fallen out 935
1933 January......	Green, seeds falling out 545	Mixed, mainly green, with flower heads 470	Green, with flower heads 1570	Green, seeds falling out 790	Green, with flower heads 635	Green, seeds falling out	Green, seeds falling out 505	Mixed, mainly green, seeds falling out 500	Green, seeds falling out 315	Green, with flower heads 580	Green, with flower heads 380

A Well-Balanced Ration for Stock Rats.

By J. H. KELLERMANN, M.Sc.(Agric.), Ph.D., Agricultural Biochemist, Section of Biochemistry, Onderstepoort.

THERE is undoubtedly no branch of biology in which so much progress has been made during the last fifteen years as in the line of nutrition, but were it not for the extensive use of the albino rat (*Mus norvegicus albinus*) in America and Europe in connection with nutrition problems much of the valuable knowledge gained so far would not have been available. At present the white rat is used almost universally in most nutrition work that is of an exact and statistical nature; a distinction it certainly deserves because the rat is such an economical animal to experiment with in view of the fact that its size makes it possible to house a large number in a relatively small space, and to provide the necessary food at a low cost. Moreover, it also has a relatively short life span, becomes sexually mature at an early age, its reproductive activities are rapid, it is omnivorous and not at all fastidious with its diet which can be absolutely controlled, and what is more, it can easily be standardized both genetically and nutritionally, a point which is of considerable importance in nutrition work.

With the object of starting a pure-bred and standardized colony, twelve (eight females and four males) Wistar Rats of the London strain were imported in 1932 from the Glaxo Research Laboratory, London. At the present writing several generations have already been raised from the original imported animals. These stock rats are hardy, active and very tame. They are housed in large cages in a room that is well lighted, well ventilated, clean and electrically heated during the cold winter months. Clean shavings are used for bedding.

In looking through the literature for a good stock ration, it was impossible to select one that was quite satisfactory, in view of the fact that recent investigations on the essentials of a good diet made it clear that the normal rations of Steenbock (1923), Waddell and Steenbock (1928), Sherman and Burtis (1928), and Bills *et al.* (1931) can all be improved upon. The stock rations of Smith and Bing (1928), Moore and co-workers (1932), Coward and collaborators (1932), and Bacharach (1933) are no doubt adequate in all respects but they are not simple and uniform enough for our purpose. In this laboratory a suitable mixture containing all the necessary ingredients is preferred to their diets which also include fresh vegetables, meat and liver, because Kon (1931) has shown that the rat is not a wise selector of different food constituents when offered separately.

The stock ration adopted in this laboratory is a modification of Waddell and Steenbocks' (1928) normal diet in so far as parts of the yellow corn and linseed oil meal have been replaced by dried beef liver, brewer's yeast and calcium carbonate. It is a dry comminuted ration with the following composition:—

Ground yellow corn	68	per cent.
Linseed oil meal	10	,,
Crude casein	5	,,
Dried brewer's yeast	5	,,
Alfalfa meal	3	,,
Butter fat	5	,,
Beef liver (dried at 70° C.)	2	,,
Bone ash *	1	,.
CaCO$_3$	0·5	.,
NaCl	0·5	,.

Fresh whole milk and tap water, fed separately, *ad libitum* daily.

The above ration has been in use now for more than a year with excellent results. 98 Per cent. of the litters weighed 40 to 60 grams at an age of 23 days. All the litters were reduced to 6 on the day of parturition as practised by Macy, Outhouse, Long and Graham (1927).

Beef liver was incorporated in the ration because it has been shown in different laboratories that liver stimulates food consumption and utilization, general growth and lactation. [Osborne and Mendel (1926), Evans and Burr (1927), Mapson (1932), Seegers and Smith (1932), Graham and Griffith (1933), Seegers and Smith (1933), and Bahrs (1933).] Whether liver owes its above qualities to its high vitamin B$_2$ content (Cuha, 1931, Graham and Griffith, 1933) or to some yet unknown essential dietary factor remains to be seen. However, Karrer and v. Euler (1933) have shown recently that a vitamin B$_2$ extract from liver possessed remarkable growth-promoting power when fed to rats. Nevertheless, by the addition of 5 per cent. brewer's yeast, the above ration is also well supplied with the vitamin B complex. The addition of yeast was considered necessary in view of the fact that the results of Macy *et al.* (1927), Evans and Burr (1928), Sure, *et al.* (1929) and Clayton (1930) show that nursing rats need an abundant supply of vitamin B if successful lactation is to take place. The yeast is obtained from the Castle Brewery, Johannesburg, in a dry commercial form. It contains 7·2 per cent. nitrogen and has been found effective in aiding growth and preventing polyneuritis when added at a 5 per cent. level to a vitamin B-free ration.

The fat soluble vitamins A, D and E are also present in optimum amounts. As a matter of fact the ration is too rich in vitamin A when the young animals are to be used for the assay of this vitamin. Of course, in assaying vitamin A it is always better to regulate first the storage of this vitamin in the aminals either by the method of Nelson (1928) or that of Garrett and Mitchell (1933) before they are put on the experimental rations. Vitamin C, although not exactly necessary in the diet of the rat, is supplied in the fresh whole milk.

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* The bone ash was prepared by ashing commercial bone meal in an electric muffle at a bright red heat until a nice white ash was obtained. The ash was then ground to a fine powder in a large iron mortar.

The alfalfa meal, in addition to containing an apparent new growth-stimulating factor as shown by Mason (1928), also serves as a good roughage.

The crude fibre (determined by Mr. Roets), ash and protein (total N-N in yeast × 6·25) contents of the ration are 4·5, 3·72 and 15·69 per cent. respectively. The nitrogen of the yeast was not calculated as protein-nitrogen in view of the fact that Still and Koch (1928) when measuring by Mitchell's method the biological values of diets containing one half of the nitrogen from yeast and one half from casein (total N – 2·9 per cent.) found little supplementary relation between the two proteins. The distribution of food energy when expressed in terms of percentages of the total energy* of the ration is: carbohydrates 68·2, fats 15·0 and protein 16·8. Approximately 65 per cent. of the protein came from plant and 35 per cent. from animal sources. It will be seen that the protein content (16·8 per cent.) of the ration falls between 14 and 18 per cent., the range in which Slonaker (1931), (1931a), (1931b), (1931c), (1931d), (1931e), obtained the best results for growth, reproduction, activity, etc., when taken as a whole. Bing and co-workers (1932) believe that the protein requirements of the mouse are certainly fulfilled by diets containing 15·6 per cent. protein (casein), and that the protein requirements of rats and mice are nearly the same.

The calcium, phosphorus and magnesium contents are 0·61, 0·44 and 0·16 per cent. respectively, with a Ca : P ratio of 1·39 which porportion has been found by Simmonds (1924), Bethke and Edgington (1927), and Bethke, Edgington and Kick (1933) to be the optimum or near optimum for the rat and for pigs. In a more recent paper by Bethke, Kick and Wilder (1932) further evidennce is given that a Ca : P ration between 2·00 and 1·00 is the most favourable for growth and bone formation in the rat. The iodine content is 33 γ per 100 grams of ration as determined by Dr. Blom (1933) whose co-operation is appreciated.

SUMMARY.

A dry comminuted ration for stock rats is described which is considered to be well balanced and adequate in all respects. It contains 68·2 carbohydrates, 15·0 fats and 16·8 protein expressed in terms of percentages of the total calories yielded by the ration. Its ash, calcium and phosphorus contents are 3·72, 0·61 and 0·44 per cent. respectively with a Ca : P ratio of 1·39.

LITERATURE CITED.

BACHARACH, A. L. (1933). Investigation into Vitamin A-free Basal Diets. *Biochem. J.*, Vol. 27, pp. 5-16.

BAHRS, A. M. (1933). The Effect of Adding Liver to the Diet upon the Growth and Reproduction of Rats. *Amer. J. Physiol.*, Vol. 105, pp. 262-265.

BETHKE, R. M., AND EDGINGTON, B. H. (1927). The Calcium-phosphorus Ratio in Animal Nutrition. *Proc. Amer. Soc. Animal Prod.*, p. 13.

* Total calories include those derived from yeast and alfalfa meal.

BETHKE, R. M., KICK, C. H. AND WILDER, W. (1932). The Effect of Calcium-phosphorus Relationship on Growth, Calcification and Blood Composition of the Rat. *J. Biol. Chem.*, Vol. 98, pp. 389-403.

BETHKE, R. M., EDGINGTON, B. H., AND KICK, C. H. (1933). Effect of the Calcium-Phosphorus Relationship of the Ration on Growth and Bone Formation in the Pig. *J. Agric. Res.*, Vol. 47, pp. 331-338.

BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. (1931). A Critique of the Line Test for Vitamin D. *J. Biol. Chem.*, Vol. 90, pp. 619-636.

BING, F. C., ADAMS, W. L., AND BOWMAN, R. O. (1932). Protein Requirements of the Albino Mouse. *J. Nutr.*, Vol. 5, pp. 571-9.

BLOM, I. J. B. (1933). Methods for the Micro-determination of Iodine in Biological Material. *O.Poort Jnl. of Vet. Sc. and An. Ind.*, Vol. 2, No. 1, pp. 123-130.

CLAYTON, M. M. (1933). The Comparative Value of Milk, Egg and Meat Proteins for Reproduction and Lactation in the Rat. *J. Nutr.*, Vol. 3, pp. 23-38.

COWARD, K H., CAMBDEN, M. R., AND LEE, E. M. (1932). A Stock Diet for Rats Bred for Vitamin Tests. *Biochem. J.*, Vol. 26, pp. 679-690.

EVANS, H. M., AND BURR, G. O. (1928). On the Amount of Vitamin B Required during Lactation. *J. Biol. Chem.*, Vol 76, pp. 263-272.

EVANS, H. M., AND BURR, G. O. (1927). A New Dietary Deficiency with Highly Purified Diets. *Proc. Soc. Exp. Biol. and Med.*, Vol. 24, pp. 740-743.

GARRETT, O. F., AND MITCHELL, H. H. (1933). An Application of the Paired Feeding Method to the Quantitative Estimation of the Relative Vitamin A Contents of Foods and Artificial Concentrates. *Am. J. Physiol.*, Vol. 104, pp. 608-614.

GRAHAM, C. E., AND GRIFFITH, W. H. (1933). Growth Factors in Liver. *J. Nutr.*, Vol. 6, pp. 179-194.

GUHA, B. C. (1931). The Sources, Stability and Chemistry of Vitamin B₂. *Biochem. J.*, Vol. 25, pp. 945-959.

KARRER, P., AND v. EULER, H. (1933). *Arkiv. Kuni., Mineral Geol.* 11n, Heft 16, 4 pp.; *Chem. Abst.*, Vol. 27, p. 5100.

KON, S. K. (1931). The Self-selection of Food Constituents by the Rat. *Biochem. J.*, Vol. 25, pp. 473-481.

MACY, I. G., OUTHOUSE, J., LONG, M. L. AND GRAHAM, A. (1927). Technique Employed in Vitamin Studies. *J. Biol. Chem.*, Vol. 73, pp. 153-174.

MAPSON, L. W. (1932). Evidence of the Existence of a Dietary Principle Stimulating General Growth and Lactation. *Biochem. J.*, Vol. 26, pp. 970-986.

MASON, K. E. (1928). The Effect of Purified Diets, and their Modifications on Growth and Testicular Degeneration in Male Rats. *J. Nutr.*, Vol. 1, pp. 311-334.

MOORE, C. U., PLYMATE, H. B., ANDREW, B. J., AND WHITE, V. (1932). Statistical Comparison of Small and Large Litters of Rats on a Normal Stock Diet. *Am. J. Physiol.*, Vol. 102, pp. 566-572.

NELSON, E. M. (1928). Regulating the Storage of Vitamin A in Animals that are to be Used for the Determination of this Vitamin. *Science*, Vol. 68, p. 212.

OSBORNE, T. B., AND MENDEL, L. B. (1926). The Relation of the Rate of Growth to Diet. *J. Biol. Chem.*, Vol. 69, pp. 661-673.

SEEGERS, W. H., AND SMITH, H. G. (1932). Influence of Raw and Whole Dried Liver on Food Consumption and Utilization. *Proc. Soc. Exp. Biol. and Med.*, Vol. 30, pp. 365-6.

SEEGERS, W. H., AND SMITH, H. G. (1933). The Presence of a New Dietary Principle in Liver. *Proc. Amer. Soc. Biol. Chem.*, Vol. 8, p. 87; *J. Biol. Chem.*, Vol. 100, p. 87.

SHERMAN, H. C., AND BURTIS, M. P. (1928). Factors Affecting the Accuracy of the Quantitative Determination of Vitamin A. *J. Biol. Chem.*, Vol. 78, pp. 671-680.

SIMMONDS, N. (1924). Observations on Reproduction and Rearing of Young by the Rat as Influenced by Diet. *Amer. Jour. Hyg.*, Vol. 4, pp. 1-108.

SLONAKER, J. R. The Effect of Different Per Cents. of Protein in the Diet:
(1931)   I. Growth. *Am. J. Physiol.*, Vol. 96, pp. 547-556.
(1931A)   II. Spontaneous Activity. *Ibid.*, pp. 557-561.
(1931B)   III. Intake and Expenditure of Energy. *Am. J. Physiol.*, Vol. 97, pp. 15-21.
(1931C)   IV. Reproduction. *Ibid.*, pp. 322-328.
(1931D)   V. The Offspring. *Ibid.*, pp. 573-580.
(1931E)   VI. Weight of Mothers during Gestation and Lactation. *Ibid.*, pp. 626-634.

SMITH, A. H., AND BING, F. C. (1928). Improved Rate of Growth of Stock Albino Rats. *J. Nutr.*, Vol. 1, pp. 179-189.

STEENBOCK, H. (1923). A Satisfactory Ration for Stock Rats. *Science*, Vol. 58, pp. 449-450.

STILL, E. U., AND KOCH, F. C. (1928). The Biological Value of Yeast Proteins for the Rat. *Am. J. Physiol.*, Vol. 87, pp. 225-248.

SURE, B., WALKER, D. J., AND STUART, E. H. (1929). A Quantitative Biological Method for the Study of Vitamin B Requirements of Nursing Young of the Albino Rat. *J. Nutr.*, Vol. 1, pp. 139-153.

WADDELL, J., AND STEENBOCK, H. (1928). The Destruction of Vitamin E in a Ration Composed of Natural and Varied Foodstuffs. *J. Biol. Chem.*, Vol. 80, pp. 431-442.

Section III.

Physiology.

Studies in Sex Physiology, No. 10 :*
The Situation of the Developing Foetus in the Merino Sheep.

By

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INTRODUCTION.

WITH regard to the situation of the foetus *in utero* of any species, there is very little definite information available, excepting, of course, shortly before birth when the dorso-sacral position generally accompanies a longitudinal cephalic presentation. Indeed Williams, W. L. (1931, p. 136) quotes figures indicating " that the probability of dystokia in posterior as compared to anterior presentation is 60 : 14 ". He adds: " posterior presentation has been common in dystokia in both mares and cows, but usually the foetus has been dead or clearly and definitely diseased. The more the problem is studied, the more evident it seems that the great majority of caudal presentations are pathological ".

Obviously the most suitable time to gain this knowledge would be during life; but as facilities existed for examining the gravid uteri taken from Merino ewes killed in experiment by Quinlan, Maré and Roux (1932a)† use was made of the available material. See Table. Although Williams, W. L. (1931) warns one that " the *position* of the foetus in a dead pregnant female *may* be quite unlike that of the living animal . . . " yet it does not appear likely that the *presentation* would be at all altered, i.e. it would be longitudinal and either the cephalic or the caudal end would be directed towards the *canalis cervicis uteri*. With regard to the *position* of the foetus, i.e. the relation between any selected point of the foetal body, e.g. the dorsum and the maternal body, e.g., vertebrae, pubis, right iliac region or left iliac region, and *posture*, i.e. the arrangement of the head, neck, and limbs, it is probable alterations may take place after death.

* For footnote, see next page.

† This experiment, undertaken at Grootfontein School of Agriculture, Cape Province, was to determine the stage during oestrus when " motile sperms in the genitalia of the ewe are capable of fertilising an available ovum ".

* For convenience this paper is No. 10 of the *Series of Studies on Sex Physiology* issued from Onderstepoort. Previous numbers are:—

1. QUINLAN, J. (1928). Vasectomy as a Method of Sterilising Ram Lambs. A Comparison with Castration. *Jl. Agric. Sc.*, Vol. 18, 26/7/28, pp. 446-459. Also published in 13*th* and 14*th Reports Dir. Vet. Educ. & Res.*, pp. 583-595.

2. KUPFER, M. (1928). The Sexual Cycle of Female Domesticated Mammals. 13*th* & 14*th Rpts. Dir. Vet. Educ. & Res.*, pp. 1213-1253.

3. QUINLAN, J. (1930). Gland-grafting in Merino Sheep. Preliminary Observations on its Influence (*a*) on Body Development, Wool Production and Progeny and (*b*) on Senility. 16*th Rept. Dir. Vet. Serv. & Animal Industry*, pp. 367-413.

4. QUINLAN, J., AND MARAIS, I. P. (1931). Gland-grafting in Merino Sheep. Preliminary Observations on its Influence: (*c*) on Castrated Sheep. *Jl. S.A. Vet. Med. Assn.* II (2), pp. 104-115. Also published in 18*th Rpt. Dir. Vet. Serv. & Animal Industry*, pp. 831-879.

5. QUINLAN, J., AND MARE, G. S. (1931). The Physiological Changes in the Ovary of the Merino Sheep in South Africa and their Practical Application in Breeding. 17*th Rpt. Dir. Vet. Ser. & Animal Industry*, pp. 663-707.

6. QUINLAN, J., AND MARE, G. S. (1930). The Hand-serving Method of Mating Merino Sheep. *Farming in South Africa*, Vol. V, No. 52.

7. QUINLAN, J. (1932). The Vitality of the Spermatozoa and the Liberated Ovum in Domestic Animals, with Special Reference to the Relation of the Time of Copulation during Oestrus to Conception. *Jl. S.A. Vet. Med. Assn.*, Vol. 3, No. 1, pp. 1-7.

8. QUINLAN, J., AND MARE, G. S., AND ROUX, L. L. (1932). The Vitality of Spermatozoa in the Genital Part of the Merino Ewe, with Special Reference to its Practical Application in Breeding. 18*th Rpt. Dir. Vet. Ser. & Animal Industry*, pp. 831-871.

9. QUINLAN, J., MARE, G. S., AND ROUX L. L. (1932). A Study of the Duration of Motility of Spermatozoa in the Different Divisions of the Reproductive Tract of the Merino Ewe. *Jl. S.A. Vet. Med. Assn.* Vol. 3, No. 4, pp. 149-162. Also published in the *Onderstepoort Journal of Vet. Sc. and An. Ind.*, Vol. 1, No. 1.

LITERATURE.

In regard to the position of the foetus *in utero* Craig (1930), merely states that this . . . " is very nearly constant in the same species, and this relation it retains . . . until near parturition, when it is changed . . . ". In regard to the *horse*, he quotes Colin, who states that " towards the termination of pregnancy the foetus of the mare lies with the belly upwards, the hind limb in the largest of the uterine cornua, and the anterior limbs and head directed towards the cervix ". Concerning the *ox*, again quoting Colin, he adds: " The belly of the foetus of ruminants is directed downwards . . . and the head is directed *backwards* ". Nothing definite is stated with regard to the *sheep*.

A significant statement is the following: " The position of the foetus towards the termination of gestation may vary occasionally, *and even frequently*, owing to the active reflex movements which it performs, and to those energetic movements, it cannot be doubted, are due the difficult presentations which the foetus offers so frequently at birth ".

Smith (1921); while repeating, for the *horse*, the above description given by Colin, adds (quoting from Ellenberger's " Physiologie "—after Franck—) that " preparatory to birth the foetus changes position and turns on its side, so as to assume, first a lateral position, and lastly an upright one, by which the foetal and maternal spines are brought nearer together. To assume this position the foetus has had to make a complete (half) revolution ". Regarding the *cow* Smith states that " the foetus lies on its back . . . as in the mare, but somewhat crooked—viz. the head inclining towards one side and the hind extremities towards the other; in all other respects its position resembles that of the foetus of the mare ".

In regard to the active movements of the foetus in late pregnancy, Smith adds: " the alteration in the position of the foetus does not occur through its own movement, but by the contraction of the uterus; on the other hand, the stretching of the limbs is the result of foetal movement ".

Williams, W L (1931), after explaining that " the foetus of unipara constitutes an arc of a circle " to correspond with the lesser curvature of the uterus, strongly contests the view expressed above, especially in regard to the dorso-pubic position alleged to be assumed by the equine foetus. He adds: " If the . . . position is physiological, it would be of great interest to discover the forces which bring about the rotation at birth "! Concerning the bovine foetus, he considers that as both foetus and uterus are so distinctly arciform, " it is very doubtful that the harmony of the two arcs should become seriously disturbed during the later stages of gestation ".

From the above references, it is clear that no precise knowledge is available concerning not only the sheep, but also the horse and cow.

MATERIAL STUDIED.

When fertility had been established by Quinlan and co-workers (1932a) in the ewes referred to above (loc. cit. footnote), the sheep were of no further use to the investigators. It was, therefore, decided to kill them at varying intervals and to preserve the internal genitalia intact for further study. Notes were kept regarding the date and hour of copulation and again concerning the hour of slaughter. The gravid uteri were removed immediately after death, sealed in tins containing 10 per cent. formalin and then despatched by rail to Onderstepoort, where, as opportunity offered, they were examined by the writers. In some instances the material had been tightly packed in the various receptacles, but in only a few cases was it difficult to arrange each specimen in the dorso-ventral position (with the *pars indivisa* of the uterus directed caudally) for photographing. It will be observed (see Plate) that the specimens have been arranged according to the age of the foetuses, concerning which details can be seen in the accompanying Table.

TABLE AND COMMENTS.

I. Serial No. of foetus.	II. Official No. of ewe.	III. No. of Table, in Experiment 2b—Quinlan, Maré, and Roux (1932a).	IV. Age of foetus. Days.	Hrs.	V. Approx. total weight of un-opened uterus. Gm.	VI. Approx. weight of foetus. Gm.	VII. Approx. size C.R. length. Cm.
1	O. 125	8 i.e. served at 18th hour of oestrus	33	4	120	1·132	2·1
2	O. 66	14 ,, ,, 36th ,, ,,	33	20	180	1·561	2·3
3	O. 136	10 ,, ,, 24th ,, ,,	35	1	120	1·926	2·4
4	O. 70	12 ,, ,, 30th ,, ,,	36	23	100	1·825	2·5
5	O. 91	2 ,, ,, onset ,, ,,	37	23	140	—	Twins 2·6 2·6
6	O. 163	4 ,, ,, 6th ,, ,,	39	1	300	4·080	3·5
7	O. 137	6 ,, ,, 12th ,, ,,	40	8	160	4·125	3·2
8	O. 64	12 ,, ,, 30th ,, ,,	42	16	120	2·771	3·3
9	O. 138	10 ,, ,, 24th ,, ,,	43	22	440	8·062	4·1
10	O. 76	4 ,, ,, 6th ,, ,,	44	21	260	7·811	3·9
11	O. 114	8 ,, ,, 18th ,, ,,	46	6	420	13·32	5·1
12	O. 140	12 ,, ,, 30th ,, ,,	46	22	280	7·866	4·8
13	O. 67	6 ,, ,, 12th ,, ,,	48	8	280	15·06	5·3
14	O. 126	2 ,, ,, onset ,, ,,	49	1	600	17·58	5·7
15	O. 108	10 ,, ,, 24th ,, ,,	49	23	500	24·15	6·7
16	O. 75	8 ,, ,, 18th ,, ,,	51	1	600	23·85	6·7
17	O. 101	8 ,, ,, 18th ,, ,,	52	1	560	25·551	7·2
18	O. 161	2 ,, ,, onset ,, ,,	53		460	27·77	7·3
19	O. 87	10 ,, ,, 24th ,, ,,	55	4	660	36·535	7·9
20	O. 72	6 ,, ,, 12th ,, ,,	55	18	1000	—	Twins 7·5 7·3
21	E. 88	15 ,, ,, 39th ,, ,,	56	5	1320	—	Twins 8·4 8·0
22	180	13 ,, ,, 33rd ,, ,,	64	16	1000	88·48	10·5
23	O. 207	5 ,, ,, 9th ,, ,,	65	21	840	102·97	11·5
24	O. 82	5 ,, ,, 9th ,, ,,	66	18	1400	120·0	11·9
25	O. 164	5 ,, ,, 9th ,, ,,	68	15	1280	151·8	12·6
26	O. 186	11 ,, ,, 27th ,, ,,	70	17	1500	177·92	13·4
27	O. 190	5 ,, ,, 9th ,, • ,,	72	13½	1400	187·2	14·1
28	O. 168	10 ,, ,, 24th ,, ,,	80	22	1740	319·8	20·0
29	G. 115	8 ,, ,, 18th ,, ,,	82	3	2040	400·0	17·3
30	G. 118	8 ,, ,, 18th ,, ,,	82	23	1640	415·0	17·4
31	O. 152	8 ,, ,, 18th ,, ,,	83	21	3860	—	Twins 18·5 18·5
32	O. 179	12 ,, ,, 30th ,, ,,	84	17	1800	457·5	19·3
33	O. 104	11 ,, ,, 27th ,, ,,	84	19	1440	414·0	18·7
34	2397	12 ,, ,, 30th ,, ,,	86	20½	3000	355·0	16·5
35	O. 189	9 ,, ,, 21st ,, ,,	87	22	3720	—	Twins 18·5 19·8
36	175	5 ,, ,, 9th ,, ,,	96	18	2200	959·0	23·5
37	O. 94	5 ,, ,, 9th ,, ,,	100	16	2480	988·5	23·5
38	4675	5 ,, ,, 9th ,, ,,	101	15	1920	942·7	22·9
39	2398	13 ,, ,, 33rd ,, ,,	102	18	2320	1010·5	21·6
40	G. 127	9 ,, ,, 21st ,, ,,	105	2	3360	1576·0	25·5
41	G. 130	3 ,, ,, 3rd ,, ,,	108	16	2580	1216·9	23·0

The following comments must be made on the above:—

(a) *Time during oestrum when ewe was served (column III).*— This information while of the greatest importance to Quinlan and co-workers (1932a), being in fact, the object of their investigations, (inasmuch as it was desired to ascertain at which stage copulation would be most successful), is only of interest in this study in connection with the ageing of the foetus.

(b) *Age of foetus (column IV).*—In his notes regarding the foetuses, kindly furnished by Mr. G. S. Maré of Grootfontein School of Agriculture, the age has been given as from the time of copulation until slaughter. While for practical purposes this is convenient, it must be borne in mind that the precise prenatal age should be dated from fertilization. Factors which accordingly should be considered are:—(a) time of ovulation, (b) time taken for ovum to reach Fallopian tube, and (c) fertilizable period of ovum. The male factors are (i) time of service, (ii) time taken for spermatozoa to meet the ovum, and (iii) longevity of spermatozoa.

Quinlan and Maré (1931) have indicated that the time of ovulation " rarely takes place before the 36th to the 40th hour of oestrus ", but nothing definite is known concerning the period taken for the ovum to reach the oviduct. In connection with this, Quinlan and co-workers (1932a) assume " a period of a few hours to enter the Fallopian tube ". Regarding the fertilizable period of the ovum, Quinlan puts this down as not even " 6-12 hours after follicular rupture " in " at least 50 per cent. of cases " (Quinlan 1932b).

Regarding the sperm cell, Quinlan and co-workers (1932a) are of the opinion that " the spermatozoa . . . are capable of . . . impregnating an available ovum from the onset of oestrus until 30 hours afterwards ". Further, that " spermatozoa may reach the abdominal extremity of the Fallopian tubes within 6 hours following coitus "; and finally, that the longevity of sperms depends on the division of the genital tract in which they are present, e.g. about 12 hours in the vagina " and up to the 48th hour after coitus " in the cervix. See No. 9 of the Series of Studies on Sex Physiology.

It is, therefore, obvious from the above considerations that the prenatal age depends on many factors, and although it may be, for practical purposes, calculated from copulation, yet strictly speaking, other factors should be borne in mind.

(c) *Weight of uterus and foetus (columns V and VI).*—Obviously this can only be approximate owing to the specimens having been placed in preservative.

(d) *Size of foetus (column VII).*—Here again only the approximate size can be given, for apart from the action of the preservative, some degree of distortion had taken place through tight packing in despatch.

It is emphasized that far more accurate observations could be made with fresh material.

DISCUSSION ON PLATE.

As the situation of the foetuses is clearly shown in the Plate, it is only necessary to state as follows:—

(a) *Twin Pregnancies.*—Of 41 pregnancies 5 were double, but of these in one case a foetus had been disturbed (No. 5). Those to receive consideration will therefore be Nos. 20, 21, 31 and 35.

(b) *Single Pregnancies.*—Of 41 pregnancies, 36 were single. Three, however (Nos. 8, 12, and 13), had been disturbed, leaving 33 for further study.

(a) TWIN PREGNANCIES.

In regard to *presentation*, all were longitudinal, the direction being one cranial (No. 31), one caudal (No. 20), and two having one foetus cranial (right horn in both instances), and the second foetus caudal (Nos. 21 and 35). As to *position*, the dorso-iliac predominated, 6 foetuses of the 7 being arranged dorsum laterally, and only one (No. 31) dorsum medially. The eighth foetus (No. 35) was dorsosacral in position. In connection with *posture* this was normal in all cases, except for the foetus occupying the left horn of No. 35. Here the head was turned backwards and it is possible that tight packing was responsible.

(b) SINGLE PREGNANCIES.

In connection with *presentation*, of 33 specimens all were longitudinal, 20 being cranial and 13 caudal. According to Williams, W. L. (1931) " in uniparous animals in *advanced* pregnancy the physiological rule is that the cephalic end of the foetus be directed towards the cervix ". The figures quoted are 99 per cent. (Schmaltz citing Kehrer) for the mare, and 95–96 per cent. for the cow.

In this series of uteri, the majority of pregnancies are not advanced. Assuming, therefore, Williams is correct, then it would appear, if the Merino sheep resembles the horse and cow, that during intra-uterine development many of the presentations now caudal would become cranial.

In regard to *position*, the following were the relations.—12 dorso-sacral, this being the case especially towards the end of the series; and 20 dorso-iliac, 10 being directed towards the right and a like number towards the left. As would be expected this series characterized the first half of pregnancy. Only one foetus was found in the dorso-pubic position.

Regarding *posture*, the general flexed condition of the head and limbs was maintained. In a few cases there were departures from the normal, brought about no doubt to some extent by tight packing.

CONCLUSIONS.

An investigation into the situation of the lamb during intra-uterine life, admittedly based on *dead* material, brings to light the following facts : —

(a) That not only in the ewe but also in the mare and cow the situation of the developing foetus is not known with any certainty.

(b) That examination of gravid uteri of the dead sheep indicates (i) longitudinal presentation apparently remains the same, whereas (ii) position and posture are likely to be changed, the former especially in early pregnancy and the latter in late pregnancy. And

(c) That of 36 single pregnancies (see Plate) 21 foetuses were placed in right uterine horn and 15 foetuses were on the left side.

The next study will deal with the relationship between the pregnant horn and the corresponding corpus luteum verum, which followed the ovulation preceding the pregnancy in question.

ACKNOWLEDGMENT.

REFERENCES.

CRAIG, J. F. (1930). *Fleming's Veterinary Obstetrics*, 4th Edit., p. 106. Bailliere, Tindall & Cox, London.

QUINLAN, J., AND MARE, G. S. (1931). The Physiological Changes in the Ovary of the Merino Sheep in South Africa and their Practical Application in Breeding. 17*th Rept. Dir. Vet. Serv. and Anim. Indust.*, p. 679. The Govt. Printer, Pretoria.

QUINLAN, J., MARE, G. S., AND ROUX, L. L. (1932a). The Vitality of the Spermatozoon in the Genital Tract of the Merino Ewe, with Special Reference to its Practical Application in Breeding. 18*th Rpt. Dir. Vet. Serv.*, Part II, p. 851-866. The Govt. Printer, Pretoria.

QUINLAN, J. (1932b). The Vitality of the Spermatozoon and the Liberated Ovum in Domestic Animals, with Special Reference to the Relation of the Time of Copulation during Oestrus to Conception. *Jl. S.A. Vet. Med. Assn.*, Vol. 3, No. 1, p. 6.

SMITH, F. (1920). *A Manual of Veterinary Physiology*, p. 784. Bailliere, Tindall & Cox, London.

WILLIAMS, W. L. (1931). *Veterinary Obstetrics*, pp. 136-138. Published by the Author, Ithaca, N.Y.

Section IV.

Poisonous Plants.

Chase Valley Disease. *Cestrum Laevigatum* Schlecht, its Toxic Effects on Ruminants.

By J. A. THORBURN, B.V.Sc.(S.A.), Veterinary Research Officer, Allerton Laboratory.

HISTORY OF DISEASE.

For years stock-owners of Chase Valley, situated on the outskirts of Maritzburg, have complained of losses in cattle from a disease which became known as the Chase Valley Disease. Losses were heaviest during the winter months, June and July in particular, the percentage mortality being affected by the nature of the season. As frequent enquiries had been made to the Department concerning the toxicity of the Inkberry plant (*Cestrum*) by stock-owners at East London, Durban and the South Coast of Natal, and as the plant is also found in the Chase Valley, suspicion naturally pointed to this plant as a possible causal agent of the disease. In the past numerous feeding experiments were conducted with specimens of this plant sent in from the above-named localities, but these yielded no definite results.

Towards the end of May, 1929, a Mr. Campbell, farming on the outskirts of the Laboratory grounds, reported the death of a beast from this disease. On post-mortem the lesions indicated death due to some vegetable poisoning. On comparison with the recorded post-mortems held by our officials on the disease, these lesions were found to be identical. Being interested the writer got in touch with Mr. Bayer of the Natal University College, who in the past had been of great assistance to Mr. Mitchell in his botanical work, and we arranged to survey Mr. Campbell's farm for possible poisonous flora. This was done next day and it was found that both this Inkberry Plant and a *Senecio* species had recently been eaten by cattle. These two plants were also found to be present in a certain paddock in the Allerton Laboratory grounds, a paddock definitely known to be dangerous to cattle during the months of June and July. This seemed to indicate that one of these two plants may be the causal agent of the disease. Material was then collected from the two plants and feeding experiments conducted.

One beast was fed on the *Senecio* plant with negative results. A second beast, No. 504, was fed on the Inkberry Plant which produced poisoning and subsequent death. This beast was well suited for this experiment as it was a young animal that had been exclusively stall fed. It was given a mixture of green berries and leaves, supplemented with hay, and allowed out into a gravelled paddock during the day.

The experiment was started on the 21st May. From the 21st until the 27th May it received 1 lb. of this mixture every alternate day and from the 27th May until the 2nd June (date of death) every day. On the 31st May, the beast was noticed to be off colour, but appeared slightly brighter the next morning. The day after the beast went down, showing marked symptoms of Chase Valley Disease, and died that afternoon. Post-mortem findings compared with those of Mr. Campbell's beast as well as those recorded by the Laboratory officials on this Chase Valley Disease. In this experiment the beast took readily to the plant and in all received 10 lb., death occurring within 13 days of commencement of the experiment.

The result of this test prompted a series of feeding experiments on a further 11 head of cattle, to be conducted to ascertain which parts of the Inkberry Plant were toxic. Green berries and leaves were separately fed and both proved toxic to cattle. Thus in the 12 animals fed on the Inkberry Plant typical cases of Chase Valley Disease were produced.

At the same time tests were conducted on a horse, a goat, 12 sheep, 2 pigs, rabbits, guinea-pigs and fowls. The plant was found to be highly toxic to the sheep and the goat but harmless to the horse, pig, rabbit, guinea-pig and fowl.

DESCRIPTION OF PLANT, *Cestrum Laevigatum*, NAT. HERB. No. 8262, BY MR. A. O. D. MOGG, DIVISION OF PLANT INDUSTRY.

Habit.—A much-branched glabrous shrub 6–15 m. high, branching from the base; stems 2·5–7·5 cm. in diameter, covered with a thin light brownish-grey bark which is easily bruised when it shows green beneath and emits an unpleasant dolorous odour.

Leaves.—Aggregated chiefly towards the ends of the branches (when growing typically as a forest sub-shrub), simple, alternate, dark green, sub-membranous, from 3 cm. by 1 cm. to nearly 10 cm. by 3·8 cm. (especially on innovation shoots or when grown as a hedge) elliptic-lanceolate, entire, obtuse at the apex, acute at the base, strongly pinnately veined, glabrous, easily bruised when the same pungent odour is emitted; petiole 0·6–1 cm. in length.

Inflorescence.—An axillary sub-spicate cymose cluster; peduncle short, 0·5–2·5 cm. long, often bearing reduced leaves with solitary or 2-nate almost sessile flowers arising in their axils and terminated by a cluster of 2-4 sessile flowers.

Flowers.—Light green with creamy-yellow corolla lobes, somewhat sweet scented.

Calyx.—Five-lobed, campanulate, valvate, glabrous, persistent, very slightly accrescent in the fruit, becoming widely companulate, green in the flower turning olive-brown in the fruit; tube 2·5 mm. long; lobes at most ¼ of that length, obtuse or truncate with scarious margins.

Corolla.—Tubular 1·5 cm. long, straight, narrowed to the base around the ovary, but shortly and suddenly dilated at the throat, 10-nerved, glabrous, lobes five, connivent, 0·5 cm. by 2 mm long, obtuse, with induplicate-valvate margins.

Stamens.—Five, included, all perfect, alternating with the corolla
lobes; filaments filiform, attached to the corolla-tube throughout
the lower two-thirds of its length, thickened near the base,
glabrous, with the free portions 0·5 cm. long; anthers short,
ovate; thecae parallel, brown when mature.

Disc.—Inconspicuous.

Ovary.—Two-chambered, shortly stipitate; style simple, filiform;
stigma greenish, capitate, dilated, slightly two-lobed, glabrous;
ovules 3–6 in each chamber.

Fruit.—A succulent indehiscent ovoid purplish-black berry 1 cm.
long by 0·5 cm. in diameter, usually with six seeds.

Seeds.—Greyish-brown, 0–5 cm. long by 2 mm. in diameter, irregu-
larly flattened and angled by mutual pressure; embryo slightly
curved; cotyledons oblong, broader than the radicle.

As already stated the Inkberry Plant is a species of *Cestrum*.
Botanical opinion for some time could not definitely determine its
exact specific name. However, Dr. E. P. Phillips, Principal
Botanist, Pretoria, forwarded a series of specimens and photographs
of the plant to Kew Gardens, and Mr. N. Y. Sandwith examined
them and definitely classified the species as *Cestrum laevigatum*.
This classification agreed with the one given by Miss H. Forbes of
the Natal Herbarium to whom specimens were also forwarded.

The difficulty of classification may have been due to the fact
that this plant is not indigenous to Natal, but originally was a
native of Tropical America. It may thus be a case of an unstable
genetic form of one species which by adapation to its local conditions
and depending on its stage of growth, assumes slight botanical
differences. This is readily seen when one compares the type of
growth of the hedge variety with that of the plant growing under
natural veld conditions, as seen in the Chase Valley. The Inkberry
Plant may be described as a sub-tropical evergreen shrub which
under natural conditions may attain a height of 20 feet and grows
on the slopes and gulleys around Chase Valley.

When used as a hedge or windbreak, by repeated cuttings it
forms a very dense bush, serving well for the purpose for which it
is used.

In the young stage the leaves are broad and long, and deep
green in colour. This is well seen from the hedge type and on the
young shoots given off from the parent tree. As the plant develops
and attains its tree-like proportions, the leaves seen on the older
branches are smaller, narrower and yellow green in colour. It is on
these that the flowers and later the berries develop. The berries are
at first a deep green in colour, which later change to deep black as
ripening occurs. The plant usually bears its flowers and berries
during the months of June and July, depending on the stage of
development of each plant and on the nature of each season.

STAGES OF TOXICITY OF THE PLANT.

From experiments conducted the results seem definitely to indicate that it is the young shoots with their broad green leaves as well as the freshly formed green berries that are the most toxic to ruminants. That is the reason why the Inkberry hedges and windbreaks have been found to be such a menace because they, in the main, are composed of such shoots. These shoots are equally toxic whether in the green stage or cut and dried, for in the process of drying it does not lose its toxicity. It is of interest to note here, that when cattle were fed on material collected from a plant, that had already formed ripe black berries, this material could be fed *ad lib* to ruminants without producing any harmful effects.

This led the writer to think that once black berries had appeared on this plant it was an indication that this plant had reached its maturity stage for that year. In this stage the poisonous properties had by metabolic processes become neutralized and rendered inert. This probably explains why previous experiments with this plant yielded no definite results, and that when such experiments were conducted, the material supplied must have been supplied from a plant in the blackberry stage.

QUANTITY OF PLANT NECESSARY TO PRODUCE POISONING.

The object was to prove definitely that this plant was the cause of the Chase Valley Disease, and in all experiments the animals were fed right up to the time that they showed first symptoms. An animal has been killed with 10 lb. of mixed berries and leaves, but there is a strong suspicion that a smaller amount of the toxic material will be sufficient.

SEASON OF THE YEAR.

Chase Valley Disease occurs mainly in the months of June and July, because it is at this time that the grazing is poor, the plant attractive and green and in its toxic stage. Cattle take quite readily to this plant despite its rather unpleasant odour, but sheep and goats will not touch it, even after being starved for some time.

ANIMALS AFFECTED.

Under field conditions the great danger is to cattle. Under artificial conditions by drenching and forced feeding small quantities of this plant prove deadly to sheep and goats. The horse, pig, rabbit, guinea-pig, and fowl apparently suffer no ill effects.

SYMPTOMS OF THE DISEASE.

The period of time from commencement of the feeding test until onset of the first symptoms varies in length, depending on the quantity as well as the toxicity of the material fed.

From the onset of first symptoms until the time of death, it is usually a matter of a few hours in most cases.

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Cattle.—In the peracute form the animal is usually found dead. In the acute form symptoms appear suddenly and are severe from the onset. They are: Increased salivation with dribbling from the mouth, running of the eyes, staring coat, arched back, extended head, cessation of rumination, loss of appetite, rapid emaciation and weakness, staggering gait, in co-ordination of movements. The eyeballs become sunken, the eyes having a staring, glassy wild look. The mucosa becomes slightly injected, moist and yellow-tinged. The animal becomes constipated and the anal mucosa inflamed. There is frequent micturition, the urine being light coloured and slightly yellow. The pulse becomes weak, the respirations shallow and increased.

In this stage when the animal is running loose, the farmer is warned to be careful when approaching and handling such an animal, because it is liable to turn vicious.

As the symptoms become progressively worse, the animal lies down and can be made to move only with difficulty. Eventually it is even too weak to do this and when in the recumbent position its head is usually held arched towards the right flank. Acute abdominal pain is shown early and this becomes progressively more severe. The animal continually grinds its teeth and groans and by restless movements tries to kick and horn its abdomen.

Just before death supervenes the animal struggles and kicks as if in terrific pain and death seems to come as a welcome relief.

In the early stages the temperature may show slight fluctuation but there is no well defined febrile reaction. From onset of first symptoms the extremities of the animal are cold and from this onset until death the time is very short, varying from 4–12 hours.

In the chronic form the symptoms are similar but less severe and more prolonged. The animal may live as long as 3–4 days and in some cases it may recover.

Recovery in such cases is of a very slow nature; the animal always remains a bad doer.

Sheep and Goats.—In these animals the symptoms appear suddenly and are very severe. The animal refuses food, discharges from the eyes, shows increased salivation and lachrymation, rapidly becomes weaker and lies down. It breathes rapidly with shallow respirations, the pulse becoming weak and wiry. The eyeballs become sunken and the pupils of the eyes dilated, giving the eyes a glassy fixed look. Urination increases and the animal suffers acute abdominal pain. Just before death occurs the animal struggles violently. From onset of first symptoms until time of death it is a matter of a few hours.

DIAGNOSIS.

This is made from the symptomatology of the disease, the time of the year, and the presence of the plant in its toxic stage, coupled with the post-mortem findings indicative of acute vegetable poisoning.

PATHOLOGICAL FINDINGS.

The extent and intensity of the pathological findings which are shown on post-mortem, depend largely on the amount of toxic material fed, over what period fed, and the duration of the symptoms produced.

They are in the main : Generalized lymphadenitis and cyanosis. The heart shows increased fluid in the pericardial sac (hydropericard.) Presence of epicardial, endocardial and myocardial haemorrhages with degeneration of the myocard. Slight increase of fluid in the thoracic cavity is seen (hydrothorax). There is a large increase of fluid in the abdominal cavity (ascites.) The liver shows subcapsular haemorrhages. Depending on the amount of plant fed, the length of time during which feeding is conducted, duration of symptoms, so the liver changes vary from acute congestion to acute inflammation and fatty degeneration to liver cirrhosis. The mucosa of the gall-bladder is thickened and oedematous and shows the presence of petechiae. The kidneys show acute congestion and degeneration. The urinary bladder is distended with straw coloured fluid. The mucosa is slightly thickened, showing presence of numerous petechiae. Alimentary tract : Abomasum shows presence of acute inflammation, walls thickened and numerous petechiae present. There is acute inflammation of the small intestines, the inflammation varying from catarrhal to catarrho-haemorrhagic. The large intestines show similar lesions. The caecum is usually filled with blood and the walls thickened and inflamed. The rectum contains hard lumps of faecal matter covered with a bloody exudate, the mucosa being markedly thickened and inflamed.

TREATMENT AND PREVENTION.

As for all vegetable poisonings, when the animal has eaten sufficient of the toxic material, treatment is of no avail. In the chronic cases that recover, the practitioner can only alleviate symptoms as they appear. This is directed towards relieving the constipation, treating the inflammation of the bowels and restoring appetite and condition. But as already stated the animals that do recover are always bad doers and from the economical point of view should be destroyed.

Prevention is directed towards keeping cattle away from localities where Inkberry grows during the time when grazing is poor and this plant is in its toxic stage, namely in June and July.

In conclusion the writer would like to emphasise that the experiments were directed towards proving that the Inkberry Plant as found around the Chase Valley is definitely toxic to cattle and is the cause of the so-called Chase Valley Disease.

ACKNOWLEDGEMENTS.

For assistance and advice on the classification of this plant thanks are due to Dr. E. P. Phillips and Mr. A. O. D. Mogg of the Botanical Division, Pretoria, as well as Miss H. Forbes of the Natal Herbarium, Durban.

For practical help and advice on Botanical Survey of Chase Valley I wish to thank Mr. A. Bayer, of the Natal University.

EXPERIMENTAL WORK.

CATTLE.

Experiment No. 1.

Period of experiment: 13 days.

Stall-fed Heifer Calf D.O.B. No. 504. *Age:* 15 months.

Object of experiment: To prove toxicity of plant.

21.5.29	1 lb. mixture of green berries and leaves fed.
23.5.29	Ditto.
25.5.29	Ditto.
27.5.29	Ditto.
28.5.29	Ditto.
29.5.29	Ditto.
30.5.29	Ditto.
31.5.29	Ditto.

Animal noticed to be off colour with slight lachrymation, salivation and increased urination.

Improved slightly in the course of the day.

1.6.29 Ditto.

Animal more or less normal.

2.6.29 Ditto.

Developed acute symptoms of cestrum poisoning.

Died in extremis at 3.30 that afternoon.

P.M. held was typical of Chase Valley Disease. P.M. No. 456.

Remarks.—Animal was stall-fed throughout. Besides receiving its cestrum allowance, it was given hay and water. During the day it was allowed out into a gravelled paddock. It took readily to the plant and took its ration up to day of death. In all it received 10 lb. of the plant. Material was obtained from Mr. Campbell's paddock.

Experiment No. 2.

Period of experiment: 37 days.

Stall-fed Bull Calf D.O.B. No. 535. *Age:* 10 months.

Object of experiment: To prove toxicity of green berries.

4.6.29-26.6.29	17 lb. berries (mainly green) fed. Material obtained from Mr. Campbell's paddock. No symptoms apparent.
26.6.29- 9.7.29	4 lb. berries fed. Material obtained from Mr. Todd's farm.
10.7.29	Animal developed acute symptoms of poisoning and died that night.

Conditions of experiment as for previous.

P.M. held was typical of Chase Valley Disease, P.M. No. 459.

Remarks.—In the early part of the experiment the material was obtained from Mr. Campbell's paddock, but towards the latter part of June it was noticed that the greater part of the material was in the blackberry stage. Suspecting at this stage of the work that the plant loses its toxicity when the blackberries form, as no symptoms of poisoning were yet apparent, the material was then collected from Mr. Todd's farm, as his bushes were still in the greenberry stage and typical cases of Chase Valley Disease had been reported on his farm.

So it was that only when material of plant in the greenberry stage from Mr. Todd's farm was fed that poisoning could be produced.

Experiment No. 3.

Period of experiment: 17 days.

Heifer D.O.B. No. 513. *Age:* 2 years.

Object of experiment: To prove toxicity of green leaves.

17.7.29	2 lb. leaves from Todd's fed.
19.7.29	ditto.
21.7.29	2 lb. leaves from Lab. paddock fed.
24.7.29	ditto.
26.7.29	ditto.
30.7.29	ditto.
31.7.29	4 lb. ditto.
1.8.29	2 lb. ditto.
2.8.29	In the morning the animal was noticed to be off colour and in the afternoon developed acute symptoms.
	Died before sundown.

P.M. typical. P.M. No. 465.

Remarks.—On examination of the bushes on Mr. Todd's farm on the 21st July it was found that the majority were in the blackberry stage. It was then decided to take material from an inkberry hedge growing around a Laboratory paddock, as it was still in the greenberry stage.

Experiment No. 4.

Period of experiment: 25 days.

Black Bull D.O.B. No. 531. *Age:* 2 years.

Object of the experiment: To prove the toxicity of the green shoots.

7.8.29–31.8.29	20 lb. of green shoots from a Laboratory paddock hedge fed.
31.8.29	Animal developed acute symptoms in the afternoon and died that night.
	From the appearance of first symptoms until time of death it was a matter of 4 hours.

P.M. typical. P.M. No. 473.

Experiment No. 5.

Period of experiment: 13 days.

Black and White Cow D.O.B. No. 534.

Object of experiment: To prove toxicity of dried leaves.

In June this animal had been fed on inkberry leaves from Mr. M. Campbell's paddock, receiving daily small doses of not more than 1 lb. From the 26th June it received similar doses of material from Mr. Todd's farm and later from the Laboratory paddock without harmful effects.

On the 18th August it was decided to test out the toxicity of a quantity of dried leaves obtained from the hedge of the Laboratory paddock. These leaves had been dried for about a month.

From the 18th until the 31st August 14 lb. of such material were fed. The dried leaves were made into a fairly hard paste with water. Quantities of 1 lb. were fed by spoon daily.

On the 31st the animal developed acute symptoms and died within a few hours.

P.M. typical. P.M. No. 474.

Similar experiments were conducted on seven more animals, feeding young leaves and green berries. Some were fed on small quantities of not more than 1 lb. daily, others on 4-5 lb. and even as much as 10 lb. weekly, at intervals with no apparent effects.

But it was noticed that in all animals when the larger dose of 4-5 lb. was fed at short intervals, it very soon produced poisoning and rapid death.

These larger quantities were made into a paste with 1 small amount of water and forcibly fed by spoon.

P.M. No.'s and D.O.B. No.'s are as follows :—

Heifer,	aged	18 months,	D.O.B. No. 501,	P.M. No. 460.
Cow	aged	——	D.O.B. No. 399,	P.M. No. 463.
Bull,	aged	3 years,	D.O.B. No. 488,	P.M. No. 464.
Cow,	aged	4 years,	D.O.B. No. 450,	P.M. No. 466.
Heifer,	aged	4 years,	D.O.B. No. 519,	P.M. No. 467.
Heifer,	aged	2 years,	D.O.B. No. 514,	P.M. No. 468.
Ox,	aged	5 years,	D.O.B. No. 443,	P.M. No. 472.

TYPICAL POST MORTEM.

Interim	Died during the night.
Rigor Mortis	Present in jaw, hind limbs, and broken forelimbs.
Condition.	Poor.
Abdomen	Not distended.
Integument	N.U.
Natural Openings	Mucous membranes, injected and moist, Anal M.M. reddened, presence of solid ingesta. Eyes sunken, pupils dilated.
Blood	Dark red in colour, stains well.
Flesh	N.U.
Subcutaneous Tissue	Small amount of fat, shows numerous red areas and gelatinous infiltration.
Peritoneal Cavity	Omentum shows numerous dark red areas, increased amount of fluid, about 500 c.c. present.
Diaphragm	N.U.
Pleural Cavities	Slight increase of fluid. Over diaphr., Pleural, Costal, Mediast. and Pericard. surfaces presence of numerous scattered red areas.
Salivary Glands	N.U.
Thyroids	Over surface presence of pinpoint dark areas, on section apparently normal.
Thymus	N.U.
Lymph Glands	Throughout carcase enlarged, on section moist with presence of red areas.
Tongue	N.U.
Pharynx	N.U.
Oesophagus	N.U.
Larynx	N.U.
Trachea	N.U.
Lungs.	N.U., except for slight injection of bronchial mucosa.
Heart..	Pericardium. There is increased amount of fluid in pericardial sac (200 c.c.).
	Epicardium. Smooth glistening and transparent with presence of numerous dark red areas particularly over the auricles.
	Endocardium. Smooth glistening and light grey in colour with presence of numerous dark red areas.
	Myocardium. Light grey in colour, with reduced consistence, and on section slightly moist with presence of small pinpoint red areas.
Liver .	Capsule smoothe and glistening with presence of irregular dark red areas seen under capsule. Its size is reduced and edges sharp. On section the cut surface is light yellow-brown in colour and lobulation indistinct. Edges are inverted and consistence increased.
	Gall-bladder is distended, with a greenish yellow thick bile ; the mucosa is thickened and shows presence of pinpoint red areas. The walls of the bladder are thickened and oedematous.
Pancreas	N.U.
Spleen	N.U.
Adrenals	Normal in size, on section is moist with presence of scattered red areas.

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Kidneys	Capsule contains slight amount of fat, strips easily leaving a greyish-red surface. On section surface is moist, and reddened zones indistinct, consistency reduced.
Alimentary Tract	Rumen contains large amount of semi-solid ingesta and the mucosa strips easily. Reticulum is filled with the ingesta and the mucosa strips easily.
	Omasum is distended with solid ingesta, and the mucosa strips easily leaving a pinkish-red surface.
	Abomasum contains a fair amount of fluid ingesta, the mucosa is swollen and shows diffuse pinkish-red surface with dark red areas. The surface is covered with a thick yellow exudate somewhat blood-stained.
	Small intestines; throughout its length shows the mucosa to be thickened and diffusely reddened as well as being covered with a yellowish-red exudate. The latter portion on external appearance has a slate-blue colour.
	Large intestines similar to small intestines. The colon has also a slate-blue colour externally.
	Caecum is filled with a dark red fluid which is ingesta admixed with blood. The mucosa is markedly thickened and reddened.
	The rectum contains hard lumps of solid ingesta which is covered with a slimy red exudate. The mucosa is thickened and diffusely reddened.
Uro-genital System	Urinary bladder is distended with a slightly turbid fluid. The mucosa is slightly thickened and reddened, showing presence of numerous small red areas.
	Sexual organs, N.U.
Nervous System	N.U.
Skeleton	N.U.
Pathological Anat. Diag	Slight cachexia. Cyanosis. Generalised lymphadenitis. Hydro-thorax, hydropericard and ascites. Numerous sub-serous haemorrhages. Sub-Epi-Endo, and Myo-cardial haemorrhages. Degeneration of Myo-card. Ascites, Cirrhosis and degeneration of liver with presence of sub-capsular haemorrhages. Catarrhal inflammation of gall-bladder. Slight hyperaemia and degeneration of kidneys. Petechiae of urinary bladder. Impaction of fore-stomachs with catarrhal abomasitis and catarrho-haemorrhagic enteritis, colitis and proctitis.
Etiological Diagnosis	Vegetable Poisoning. Feeding *Cestrum laevigatum* (Chase Valley Disease).

SHEEP.

To test out the toxicity of this plant on sheep it was fed to eleven sheep and produced toxic effect resulting in death.

It was found that even after prolonged starvation sheep would not eat of this plant. In all cases it had either to be forcibly fed by spoon or drenched with a stomach tube.

In the first experiment on sheep D.O.B. No. 613 the animal received 1 lb. of minced green berries on the 18th July and a further 21 lb. on the 19th. On the 18th the temperature rose to 103·6 F., and on the 20th to 105. On the 21st it dropped down to normal and continued normal until the 30th. On the 30th the animal received 3 lb. of minced green berries, and in the evening temperature rose to 103·4, the animal showing marked symptoms of disease. It died overnight. Post-mortem held was indicative of acute vegetable poisoning, as well as comparable with the post-mortem lesions seen in cattle dying from cestrum poisoning.

A similar experiment was done on sheep D.O.B. No. 611; only in this experiment the animal was drenched with a stomach tube with a watery extract of 3 lb. of minced green berries and leaves.

Experiment started on the 7th August and animal received the above quantity. On the 8th the temperature rose to 103·6, and animal was noticed to be off colour. On the 13th August it received a further 4 lb. of watery extract of berries and leaves. On the 14th the temperature rose to 105·2, the animal showing acute symptoms. On the 15th it died.

Post-mortem held was typical.

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An experiment was then conducted to test out the toxicity of young cestrum leaves on sheep D.O.B. No. 663. On the 6th September it was drenched with a watery extract of 4 lb. of minced green leaves. On the 7th the temperature rose to 105·4. The animal developed acute symptoms and died *in extremis* at midday.

Post-mortem typical.

A similar experiment was conducted on sheep D.O.B. No. 660, which on 6th September received a watery extract of 2 lb. of minced green leaves. On the 7th the temperature rose to 105·6. Animal developed acute symptoms and died that afternoon.

Post-mortem typical.

Similarly for sheep D.O.B. No. 658, which received 2 lb. of green leaves in the morning of the 10th September. Temperature rose to 104·4 that afternoon. Acute symptoms developed and on the following day it died.

Post-mortem typical.

Similarly for sheep D.O.B. No. 662, fed on the 17th September; death occurred within 24 hours, the temperature rising to 105·8 on day of dosing.

Post-mortem typical.

It was then decided to try a smaller quantity of green cestrum leaves. Sheep D.O.B. No. 664 was dosed with a watery extract of one and a half pounds on the 13th September. The temperature rose to 105·4 on the morning of the 14th. The animal died at noon.

Post-mortem typical.

The next object was to ascertain whether the green leaves in the dried state would be toxic to sheep. For this experiment four sheep were used.

D.O.B. No. 661–24397 received extract of 1 lb. dried leaves, producing death in 24 hours.

D.O.B. No. 659–24439 received extract of 2 lb. dried leaves, producing death in 24 hours.

D.O.B. No. 657–24525 received extract of 2 lb. dried leaves, producing death in 48 hours.

D.O.B. No. 666–24607 received extract of 3 lb. dried leaves, producing death in 12 hours.

GOAT.

In order to ascertain whether this plant would be toxic to goats, goat D.O.B. No. 609 was used.

On the 7th August, 1929, it received a watery extract of 4 lb. of minced green berries. On the 13th it received at further 4 lb. On the 22nd it received an extract of 1 lb. of berries and leaves. On the 27th it received a further 5 lb. Shortly afterwards the animal developed acute symptoms of poisoning and died on the 28th.

Post-mortem typical.

FURTHER EXPERIMENTS.

This plant was fed to the horse, pig, rabbit, fowl and guinea-pig with apparently negative results.

Fig. 1.   Branch, adult tree, showing berries, flowers, and leaves.
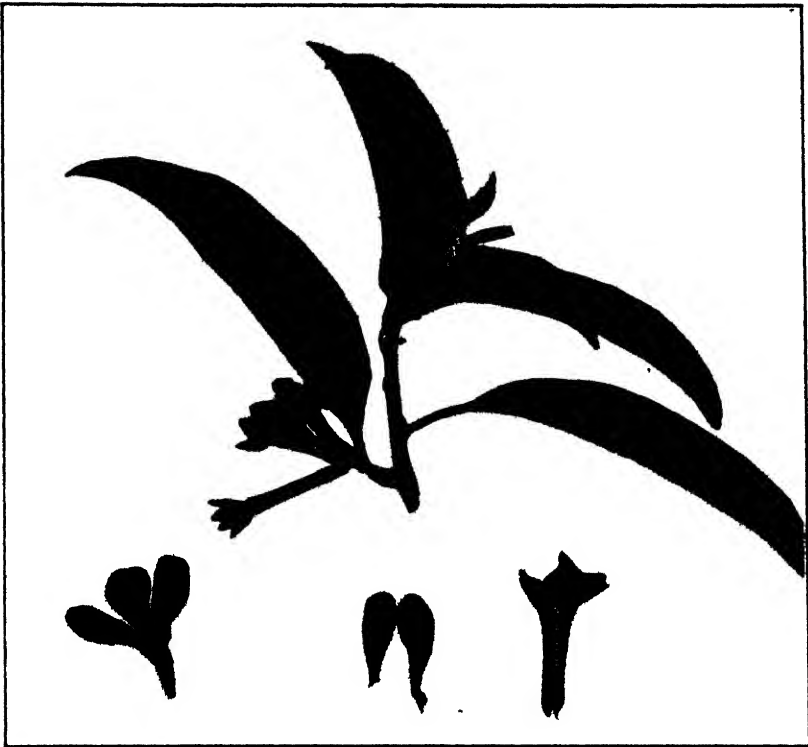
Fig. 2. Berry, leaves, and flowers, cross-section of flowers and berry.

Urginea Capitata Baker—The Berg Slangkop.
Its Toxic Effect on Ruminants.

By D. T. MITCHELL, M.R.C.V.S., formerly Veterinary Research Officer, Allerton, Natal, and

A. S. CANHAM, F.R.C.V.S., Veterinary Research Officer, Allerton, Natal.

With a Brief Botanical Survey of Area

By A. M. BAYER, M.Sc., Lecturer in Botany, Natal University College, Pietermaritzburg.

Introduction.

In my previous report on *Urginea macrocentra* Baker, that appeared in the Director's 11th and 12th Report it was mentioned that in South Africa three species of Slangkop were known to be poisonous to stock. To this list must now be added *Urginea capitata* Baker—the Berg Slangkop.

As the name implies it is found in Natal mainly in the vicinity of the Drakensberg Range. This work was done on plants obtained from the National Park area near Bergville. Steyn (1932) states it is also found in the Transvaal and Griqualand East. The plant was identified by Dr. E. P. Phillips of the Division of Botany.

DESCRIPTION OF *Urginea capitata.*

Urginea capitata Baker; bulb globose, 1¼–2 in. diam.; leaves 6–8, not fully developed till after the flowers fade, linear, a foot long, ⅓–½ in. broad; peduncle lateral, terete, ½–1 ft. long; raceme many-flowered, capitate, globuse, 1½ in. diam.; pedicles at first ¼–½ in. long, finally 1½ in. long; bracts minute, ovate, deeply saccate in the middle; perianth ⅙–1/5 in. long, white inside, bright claret-purple outside; filaments clavate, much shorter than the perianth; style tricuspidate, as long as the ovoid ovary; capsule ⅜ in. long.

HISTORY OF OUTBREAK.

On 31st October, Mr. Otto Zunkel who had taken over the National Park, sent 2 spans of oxen from his farm Needwood to this new farm and on 31st October they were placed in the " Pastures " camp. On the 1st November 144 head of mixed cattle were also placed in this camp while about 20 milk cows were drafted to the camp known as the Vemraan or Dooley camp. On 2nd November it was reported that the oxen were sick and purging profusely. On 3rd November, two oxen died in the morning and three in the afternoon, while on 4th November a further five died and a number of the mixed lot were also sick.

Mr. Zunkel then moved all his cattle, with the exception of the milk cows and their calves, back to another farm " The Lake " where deaths continued. He lost in all 44 head of stock. Blood smears were negative and chemical analysis of organs and ingesta showed nothing in the way of chemical or alkaloidal poisons.

About the middle of November Mr. Zunkel borrowed oxen from Ladysmith and Colenso and ran them with his milk cows and calves on Vemraan camp. All cattle here remained healthy.

As a result of a visit made it was seen that there was a great difference in the veld on Vemraan and Pastures camps. The former was grass veld with some low scrub and the latter open veld with many liliaceous plants, senecios and studded with Proteas. It was especially noted that *Urginea capitata* (at that time not identified) and many species of senecios occurred in much greater abundance on Pastures than on Vemraan. The Urginea showed evidence of having been browsed on fairly freely but only rarely were Senecios found eaten. The symptoms shown by the affected cattle were as follows:—tucked up appearance, sunken eyeballs, loss of appetite, no elevation of temperature, profuse diarrhoea with blood stained faeces with clots of unaltered blood in some cases. Post-mortem examination showed mild gastro-enteritis.

Mr. Zunkel placed his herd at our disposal and the following tests were arranged.

(a) One seven months' old tollie to be forcibly fed with the plant.

(b) 12 young stock (6–9 months) to be placed on Pastures.

(c) 12 young stock (12–18 months) to be placed on Pastures.

None of these cattle had had previous access to Pastures. This plan was decided upon on the 7th December when the three groups of test animals were immediately drafted to this camp. The tollie was dosed at 7 p.m. on the 7th December.

Mr. Zunkel's farm was left on 8th December when he informed me he was placing all his cattle on Pastures the next day. He also informed me that the loaned oxen after dipping on the 6th had been placed in the Pastures camp on the 7th.

Allerton Laboratory was reached on 10th December when at 10 a.m. Government Veterinary Officer Diesel rang me up and informed me that the loaned oxen in Pastures were purging badly and requested my return.

Mr. Bayer, Lecturer in Botany, Natal University College, accompanied me. I have pleasure in attaching his survey report to my article as part of it.

Mr. Bayer's report will show that the botanical evidence pointed strongly to Urginea as the suspected plant.

On arrival at the farm on the afternoon of the 11th December, 10 oxen had been purging badly but were recovering as a result of treatment while the tollie had died early that morning prior to our arrival. The post-mortem of the tollie revealed the following:— Pulmonary congestion and oedema, pericardial and endocardial extravasations, cyanosis of the mucosa of larynx, pharynx and trachea, slight gastritis, acute haemorrhagic enteritis of lower half of the ileum, acute haemorrhagic inflammation of rectum.

In the light of this it was decided to conduct feeding tests.

TOXICITY OF PLANT.

To be capable of causing so many deaths in so short a time points to the fact that the poisonous principle of the plant must be very powerful or that the plant must be present in great quantities. The natives, however, assert that buck eat freely of the plant and they themselves use the cooked leaves as an article of diet. What appears strange is the fact that the previous owner had introduced cattle frequently and at all seasons into the " Pastures " camp for the past seven years without loss. Moreover, he considered the grazing at " Pastures " the best on the farm and ran his milk cows here.

SYMPTOMS.

These under natural conditions were not numerous and are similar to what one would expect in plant poisons, viz. tucked up appearance, eyes sunken into orbital fossae, no temperature, loss of appetite, profuse diarrhoea. A rather characteristic symptom noted was excessively blood stained diarrhoea, which in some cases resembled pure coagulated blood. These are, of course, quite a different picture from what one sees in cases of *U. macrocentra* poisoning where constipation is marked. Symptoms appear quickly and then according to the severity of them the animal either lingers on or dies quickly.

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POST-MORTEM IN NATURALLY PRODUCED CASES.

From actual field observations the following changes were generally found:—

No distension of the carcase, escape of small amount of ingesta from mouth.

Mucous membranes of lips, mouth, tongue and oesophagus normal.

Trachea and lungs: Normal.

Rumen and contents: Apparently normal.

Reticulum and contents: Apparently normal.

Omasum and contents: This stomach showed either " patchy " or marked inflammation of the leaves, with ulceration of the membrane over several of the inflamed areas. The contents were rather more fluid than normal and were slightly blood stained.

Abomasum and contents: This stomach showed either acute or subacute inflammation of mucous, submucous and muscular coats, patchy in character, in some, membrane detached and stomach contained blood clot.

Small intestines and contents: Very few slight patches of inflammation—lumen filled with black viscid fluid.

Spleen: Normal.

Kidneys: Slight inflammation.

Glands: Many of these were slightly haemorrhagic but not enlarged.

Liver: Enlarged, paler than normal and presented a " cooked " appearance.

Urinary bladder: Greatly distended with normal urine, appearance of bladder normal.

EXPERIMENTAL WORK.

(a) Feeding Tests.

No previous work* done on this plant could be traced. In the experiments carried out it was found that the animals took the leaves of the plant quite readily when they were mixed with lucerne. The cattle used varied from 18 months to 4 to 5 years of age. These were constantly stabled during the experiment.

C. 442. Age: 18 months.

Fed with one pound of plant on 14th December, 1926, taken mixed with lucerne.

16th December, off feed and water; lying down most of time; faeces soft; evidence of abdominal pain.

Remarks.—This animal received 1 lb. of the plant but only developed mild symptoms. The symptoms continued until the 18th December, when indications of recovery became apparent. This animal was discharged about 2 weeks later quite recovered.

* In 1926, Mr. P. L. le Roux, late Veterinary Research Officer, Onderstepoort, informed me that he had proved the plant growing at Ermelo toxic to sheep. (D. G. Stern.)

C. 423. Age : 2 years and 4 months.

> Fed with 2 lb. of plant on 13th and 14th December, 1926, mixed with lucerne.
>
> 15th December, large quantities of fluid faeces passed; abdominal pain; animal off feed and water; lying down frequently. These symptoms persisted until 17th when the quantity of faeces became less, mixed with mucous, and blood stained. Eyes very sunken. Animal looking miserable. A second attack of purgation occurred on the 18th which persisted. Animal losing condition. A small quantity of food is now being taken.

Remarks.—This animal received 4 lb. of the plant and showed fairly marked symptoms two days after commencement of feeding. By the 3rd day symptoms were marked. No more of the plant was given and the animal gradually recovered, being discharged in about 2 weeks.

C. 427. Age : 4 to 5 years.

> Fed with 3 lb. of plant on 13th and 14th December, 1926, mixed with lucerne.
>
> 15th December, small quantity of greenish faeces passed; later grunting, off feed; evidence of great abdominal pain; paddling movements with the hind feet continuous; not lying down.

Post-mortem.

> Died about 7.30 on night of 15th December.
>
> The post-mortem showed: Cyanosis of pharyngeal mucosa; oedema and hyperaemia of lungs; Trachae: foam.
>
> Heart: Inhibition right ventricle and extravasations left ventricle.
>
> Liver: Congestion.
>
> Kidneys: Congestion of intermediate zone. Linear hyperaemia of cortex.
>
> Abomasum and duodenum: Slight patchy hyperaemia.
>
> Ileum: Acute haemorrhagic enteritis; contents blood stained.
>
> Large intestines: Acute patchy hyperaemia.
>
> Rectum: Diffuse hyperaemia with well marked longitudinal lines of acute hyperaemia; mucosa thickened and catarrhal.

Remarks.—Being an older and well grown out animal 6 lb. of the plant was fed, death resulted in two days from commencement of feeding.

C. 472. Age : 4 to 5 years.

> Fed on 13th and 14th December, 1926, with 4 lb. of plant leaves mixed with lucerne.
>
> 15th December, purging profusely large quantities of fluid faeces; no food or water taken; animal dull.
>
> 16th December, no faeces passed.
>
> 17th December, diarrhoea again commenced in the afternoon; larger quantities fluid faeces passed; eyes sunken; rapid loss of condition; abdominal pain; nasal discharge.

Post-mortem.

Death occurred on 19th December, 4 a.m.

Post-mortem showed: Cyanosis of pharyngeal mucosa.

Oedema and hyperaemia of both lungs. Foam in Tracheae.

Extravasations left heart.

Slight congestion of liver, spleen and kidneys; marked patchy hyperaemia of mucosa of abomasum, especially of folds. Acute diffuse hyperaemia of duodenum with Zebra markings. Jejunum: ditto.

Ileum: Cherry red mucosa, contents deeply blood-stained, bright brownish red, increasing in intensity to ileo-caecal valve. Caecum: ditto.

Colon: Patchy hyperaemia.

Rectum: Intense diffuse hyperaemia; mucosa thickened, covered with tenacious blood exudate. Collection of blood clots in front of anus.

Remarks.—This animal received 8 lb. of the leaves but only died in 5¼ days from the commencement of feeding.
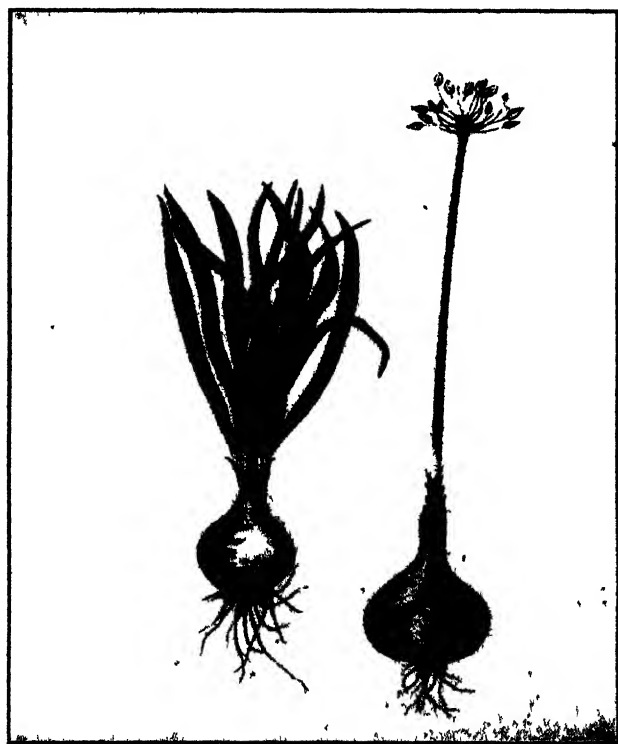
Fig. 1. *Urginea capitata* Baker, about one-third natural size.

C. 468. Age : 3 to 4 years.

> Fed on 17th December with 1 lb. of plant leaves mixed with lucerne.
>
> Symptoms shown were only mild, similar to C. 442 and this animal recovered rapidly.

(*b*) *Toxic Dose.*

From these tests it would be difficult to give an exact figure for the amount of the plant to be eaten to set up fatal cases. One would suggest that for an average sized beast the amount would be between 6 and 8 lb. From the fact that these experimentally fed animals ate the plant quite readily on two occasions when it was mixed with lucerne one could quite imagine that under field conditions animals would eat fair quantities at one time. This would rather indicate that the taste was not objectionable to them.

(*c*) *Symptoms.*

In neither of the animals that died was blood stained diarrhoea present, this was probably due to the rapidity of the poison. If they had had smaller doses and lived longer dysentery would probably have been present. No temperatures were raised during the feeding experiment.

(*d*) *Post-mortem.*

The lesions observed in these animals were almost identical with those seen in naturally occurring cases in the field.

CONCLUSIONS.

(*a*) A description of *Urginea capitata* and a survey of the area in which it was found is given.

(*b*) Symptoms and post-mortem changes as observed under both field and laboratory conditions are given.

(*c*) Results of feeding the plant to experimental animals are recorded.

(*d*) It appears as if fatal cases of poisoning by this plant can be produced by feeding from 6 to 8 lb. of *Urginea capitata*.

(*e*) The taste of this plant does not seem to be objectionable to animals as it was taken quite readily when mixed with lucerne.

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9. BOTANICAL SURVEY OF AREA.

By A. W. BAYER, M.Sc., Lecturer in Botany, Natal University College, Pietermaritzburg.

A PRELIMINARY survey of the two camps showed that they were ecologically very different in composition.

The Pastures Camp.

The Pastures camp (in which the deaths occurred) was an open community. Only in the lower parts of the camp were grasses at all abundant, and even

then they were rather sparse. Owing to grazing they were not much more than 3-4 inches high. In the upper parts owing partly to road-making operations, and also probably to increased erosion due to the steepness of the ground, the vegetation was very thin and only a few ruderal species were able to exist successfully. Amongst the grasses noticed were *Digitaria (ternata)*, *Setaria verticillata*, *Eragrostis (plana)*, *Andropogon schirensis*, *Panicum natalense*, *Aristida Galpini*, *Paspalum* sp. Associated with these were noticed *Senecio sera*, *S. speciosus*, *S. nastulatus*, *Helichrysum aureonitens*, *H. appendiculatum*, *squamosum*, *Haplocarpa scaposa*, *Eriosema* sp., *Crotolaria* sp., *Hermannia veronicifolia*, *Rhus discolor*, *Acalypha peduncularis*, *Oxalis* sp., *Cynoglossum* sp., *Scilla* spp., *Hypoxis* spp. and *Urginea capitata*.

Owing to the paucity of the grasses, the associated plants showed signs of having been eaten by the cattle. Although a careful search of the camp was made for known poisonous plants none were seen. A few plants of *Morea rivularis* were found which were near a small stream, outside the camp. These had not been touched by the cattle. *Urginea capitata* was, however, very common in the camp, and scarcely a tuft of leaves of this plant could be found that had not been cropped. A few of the *Hypoxis* spp. (of which there were common) had also been eaten off.

The Vemraan or Dooley Camp.

The Vemraan camp was typical high veld grassland. It had evidently not been strongly grazed and the grass stood 18-24 inches high. The chief grasses noted were *Themeda triandra*, which was dominant, *Monocymbrum cerisaeformis*, *Setaria (verticillata)*, *Tricholaena setifolia*, *Eragrostis brizoides* and *chalcantha*, and *Trichopterix simplex*. Amongst the associated plants the following were noticed: *Helichrysum squamosum*, *H. adenocarpum*, *Senecio sera*, *Acalypha (peduncularis)*, *Cluytia natalensis*, *Scilla* sp., *Hypoxis* sp., and a few shrubs of *Buddleia salvifolia*. These associated plants were not numerous and had evidently not been touched by the cattle. On the eastern side of the camp the composition was somewhat different, consisting of Bracken Fern societies, and containing numerous associated plants, viz., species of *Helichrysum*, *Senecio*, *Cluytia*, *Acalypha*, *Scilla*, *Hypoxis*, and *Urginea capitata*. As the grazing on this side was poor, the cattle had kept to the grasses at the top of the slope.

As a result of this survey, it was felt that the poisoning, since it was probably of vegetable origin, must be due to *Urginea capitata*. In order to make quite certain that the plant did not occur in the Vemraan camp to the same extent as in the Pastures camp, it was decided to make the survey more intensive. Accordingly, a handy quadrant was improvised out of a few yards of string and a few sharp sticks. This was easily pegged down and moved about, and enclosed an area of four square yards. At first the quadrants were mapped rather intensively, but it soon became apparent that the time at our disposal would not permit of such a procedure. It was therefore decided to concentrate upon *Urginea capitata* and the spp. of *Hypoxis*. Quadrants were taken in places where *Urginea capitata* was very common, and also where it was less frequent. These are designated respectively as maximum and minimum quadrants in Table 1 below.

TABLE 1.

	Pastures.		Vemraan.	
	Urginea.	Hypoxis.	Urginea.	Hypoxis.
Quadrant No. 1 : Maximum Quadrant...	49	6	4	nil
Quadrant No. 2 : Maximum Quadrant...	67	42	3	7
Quadrant No. 3 : Minimum Quadrant....	14	32	nil	67
Quadrant No. 4 : Minimum Quadrant....	11	43	nil	70

Several quadrants were taken in places where the distribution was medium and the average of these is given in Table 2 :—

TABLE 2.

	Pastures.		Vemraan.	
	Urginea.	Hypoxis.	Urginea.	Hypoxis.
Average quadrant......................	22	16	nil	62

These showed that in places the frequency of *Urginea capitata* was very high indeed in the Pastures camp. As many as 67 plants occurred in four square yards. In the Vemraan camp *Urginea capitata* was relatively rare. The *Hypoxis* spp. were much more frequent here than in the Pastures camp. Accordingly with the assistance of natives, kindly lent by Mr. O. Zunkel, of the National Park, a few sackfuls of *Urginea* plants were collected and brought back to the Veterinary Laboratory at Allerton for further investigation and feeding tests.

BIBLIOGRAPHY.

MITCHELL, D. T. (1927). *Urginea Macrocentra* (Baker). Its Toxic Effects on Ruminants. *11th & 12th Report of Director of Veterinary Education and Research*, Union of South Africa.

STEYN, D. G. (1932). Known Poisonous Plants in the Union of South Africa. *Jnl. of S.A. Vet. Med. Association*, Vol III, No. 4, December.

PLATE.

1. Sketch of plant showing flowering head that appears first.

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